

nature*September 16, 1976*

Still plenty for the BA to do

ANOTHER meeting of the British Association for the Advancement of Science (BA) comes and goes, and yet again the question arises, do we still need the BA, and if so, for what purpose?

Certainly, the annual meeting is not the only activity of the BA. Throughout the year there are study groups, science fairs, schools conferences, visits, publications, and so on, and it is indisputable that much of value is achieved by all this. But a lot of energy goes into the annual gathering, at which a good accumulation of speakers is assembled. To judge by the size of the audience at many of these talks, this effort is simply not being repaid by enough public interest to warrant an endless annual commitment. Is the fault with the BA and its organisers or is it elsewhere?

It would be easy to blame the BA. "Meet Britain's leading scientists" ran the headline on the poster put about locally in Lancaster. Directly beneath it was a large picture of the association's irrepressible and telegenic executive secretary, Magnus Pyke. Some of Britain's leading scientists, whose contributions are essential if the BA is to survive, might reasonably wonder whether the message should not have read, "Meet Britain's best known scientist".

The media do not help. Armed with copious preprints of lectures, correspondents find it all too easy to turn in large quantities of uncritical copy of the "said Dr Smith" type, which manages at once to convey the impression both of a lack of authority and of exaggerated claims. This conceivably does more harm than good in helping the public to a better appreciation of the achievements and limitations of science; it conceivably also scares away potential friends of the BA among the scientific community.

But it is an easy escape to blame the BA, its slightly amateurish image, its press coverage, for a major deficiency in us, the scientists. The BA still manages to persuade a good number to come and deliver lectures, but with many, no sooner is the lecture delivered than it

is a case of up and away, probably to be back in the laboratory as soon as possible. Maybe there are pressing engagements elsewhere, but perhaps we have things to learn from a diverse audience every bit as interesting as they have to learn from us. And, of course, the number of practising scientists who go to BA meetings if not invited to speak is all too small. As a result, the annual gatherings seem to lack the necessary critical mass of those actually producing the subject matter. Such a cavalier attitude towards specialist conferences would, of course, be unthinkable.

It may be argued that scientists have better things to do than spend several days adding to their general scientific knowledge. If there was much evidence that scientists used other ways of preventing themselves from getting caught in a specialist rut, then that might be a perfectly valid argument. But we are dubious that the average research scientist is anything like as well read as he or she should be to take full advantage of all the intellectual opportunities that science has to offer. And we would not be surprised if this narrowness is significantly more pronounced in the United Kingdom than it is in many other parts of the world, no doubt largely as a result of an educational system which, for better or worse, produces single-minded, specialist experts at the age of 24.

There was a time, and not too long ago, when this was something to be proud of. But with changing economic circumstances people are beginning to ask whether, without too much lowering of the standards of scholarship, it might be possible to ensure that the scientist's interests are not quite so intensely focussed. The BA should undoubtedly have a role to play in this by providing an arena in which scientists can learn from each other, from administrators, from politicians and even from taxpayers. This requires a broader commitment, of a kind which there once was, but which is now lacking. Is there any chance that Britain's scientific community might regain it in the future? □





National Air and Space Museum, Washington, DC

America's history lesson

Chris Sherwell sees the Smithsonian's latest spectacle in Washington, the National Air and Space Museum

TO glide from Washington's white heat of late summer, past the imposing pink marble and tinted window facade of the National Air and Space Museum, and on into the air-conditioned splendour of an enormous hall dedicated to the milestones of aviation, is something of an experience. For there, in just one room, is the Wright Brothers' Kitty Hawk; Lindbergh's Spirit of St. Louis; the first plane to break the sound barrier; and the X-15, which has flown at Mach 6.72. And that is just on the 'Air' side. For 'Space', and in the same room, there is a replica of Sputnik and a duplicate of Explorer I; John Glenn's Mercury capsule, Friendship 7; Gemini 4, which carried the first US space walker; a replica of the Mariner 2 interplanetary probe to Venus; the Apollo 11 command module, Columbia; and the first piece of "touchable" lunar rock. It is all a remarkable introduction to a remarkable display.

Nearly three million people have already traversed that hall, and the museum opened only eleven weeks ago; on one particular day, 82,000 people decided to visit. All seek to be informed and educated in an interesting and entertaining way about the history of aerospace. Most leave satiated. The man who piloted 'Columbia' on that memorable moon landing flight, Michael Collins, is the museum's director. He acknowledges that there is no accepted view of what a museum is supposed to do, but at his disposal is a team of 250, a vast archive, a huge 'spacearium', an enormous theatre with a 55-foot high screen, scores of audio-visual aids and, of course, hundreds of

artefacts—which means aircraft and spacecraft. The result is a 23-section history of flight, beginning with balloons, and ending with the Universe, with digressions into flight and the arts, air-traffic control and the benefits of flight on the way.

Although the balance of the museum's overall display is in favour of Air rather than Space, the most spectacular exhibits are the space-related ones. The Skylab Orbital Workshop and Multiple Docking Adaptor, together with the giant Minuteman and other missiles, dominate the second major hall, the Space Hall. Next to these are the Apollo-Soyuz spacecraft and docking module; nearby is a Lunar Module, with the Orbiter and Surveyor satellites suspended above. A room dedicated to the Moon landing project includes a complete rundown on all the crews of manned US spacecraft, a full-size mock-up of the Lunar Module cockpit, displays of experiments performed on the Moon (along with the Lunar Roving Vehicle) and several samples of lunar rock.

None of this could have happened without rockets like the Saturn V, at 353 feet so high that there is room in the museum for only one of its main engines; mirrors have to be deployed to give some idea of the overall size. Rooms on rocketry and rocket history accord due prominence to Goddard, Oberth and Tsiolkovsky, but the television series *Star Trek* is not forgotten either. The superb satellite room has com munications, Earth monitoring, science and weather satellites, but no spy satellites. Collins would like one, but no one acknowledges they exist,

and no one has recovered one.

Air sections too have their own attractions and distractions. Apart from the World War rooms, there is, for the more ghoulish, a constantly running film of the last flight of the Hindenburg and film of the more spectacular of the crazy acrobats who flew on planes rather than in them. In the Sea-Air Operations rooms visitors can stand on the bridge of an aircraft carrier and watch planes land on the deck, or alternatively (and more alarmingly, since there is no attempt to explain the military concept behind an aircraft carrier) join the pilots of the planes themselves as they go on a short 'mission'. Elsewhere, certain technical principles are exceedingly well demonstrated. There are explanations of the propeller, the turboprop engine, jet propulsion and, in the Flight Technology room, of supersonic flight and even of flight itself.

As always, the problem, even in an aerospace museum, is space—that is, the lack of it. The museum is, in fact, only two-thirds of its originally proposed size. Thus, there has to be some disappointment for everyone. In the third major hall, the Air Transportation Hall, for example, a Douglas DC-3 hangs majestically, "the most important single aircraft type in the history of air transportation". The same could not be done for what may turn out to be the most important planes since—the Lockheed C-5A and the Boeing 707. The problem, of course, is that there is no room for what would need to be another airport in the centre of Washington.

How, then, did Collins and his team decide priorities? "It's an imperfect process", he says; some categories—First and Second World War aviation, for example—chose themselves. One problem, revealed by an early survey of audiences in four of the halls, is that people by-pass important exhibits in their desire to see particular attractions like the Spirit of St. Louis. That exhibit is already the most popular, and Collins suggests that this is something to do with Lindbergh himself: people can relate to his "one-man show" a lot better than they can to the space programme, where there is "a cast of hundreds of thousands".

In fact, although the enormity of the US space effort is more than adequately conveyed by the sheer size of some of the exhibits, the impact which the aerospace industry, indeed flight itself, has had on American people and Western society generally is perhaps insufficiently emphasised. Partly, this is a problem facing any museum, where the idea of history as events, turning-points and personalities finds its greatest expression. But it is also a matter of perspective.

Take one particular personality, Howard Hughes—surely the *eminence grise* of US political and economic life. On the tiny Giants of Air Transportation exhibit the briefest of blurbs says only: "Howard Hughes for many years was the guiding force behind TWA and worked on the design of the Lockheed Constellation". In the larger Flight Technology room, the Hughes H-1 Racer is the centrepiece, and is represented, proudly, as "a major milestone on the road" to Second World War fighters like the the Grumman Hellcat, the Japanese Zero and the German Focke-Wulf FW 190. And in the Benefits of Flight room, Hughes Aircraft and the other major aerospace corporations like Boeing, Lockheed, Rockwell, Northrop, Grumman and McDonnell Douglas, merit just a mere mention on a backdrop list. Nowhere in the museum can the links, if any, between war, politics, the economy and the major corporations be more than guessed at or presumed.

Crucial decisions of the present—in the case of the US, those concerning the so-called "Sale of the Century" of fighter planes, the B-1 Bomber or the Boeing 7X7 and 7N7—receive little emphasis. There are some elements of topicality—a full-scale model of the Viking Lander, for example. But the biggest gap occurs with the supersonic transport, or SST, of which there is not even a passable model. The decision not to go ahead with this in the USA was, in the view of many, a defeat for science and technology. Not to have an exhibit is a defeat for a museum which aims to look to the future as well as the past, and which actually manages this, if somewhat disappointingly, with the Space Shuttle. Collins himself admits that this may be a weakness, and even allows that, as far as topicality is concerned, the museum "does not do that very well".

Not that the museum fails totally to overcome the problem of presenting history in terms of events. But it does go only a little way towards describing the social impact flight has had. The Benefits of Flight room is an acknowledgement that the major exhibits do not offer aerospace automatic self-justification. There the view is expressed—near a deactivated atomic bomb—that flight has contributed to peace rather than war, at least between the major powers ("the sword over Damocles' head"). Exhibits in the same room refer to work on the Earth's resources, to scientific research, to agriculture, to the weather, to communications and so on. A juke-box blaring out Elton John's "Rocket Man" and Frank Sinatra's "Come Fly with Me" spotlights the cultural impact; so does the list of additions to our vocabulary, and the whole Arts room.

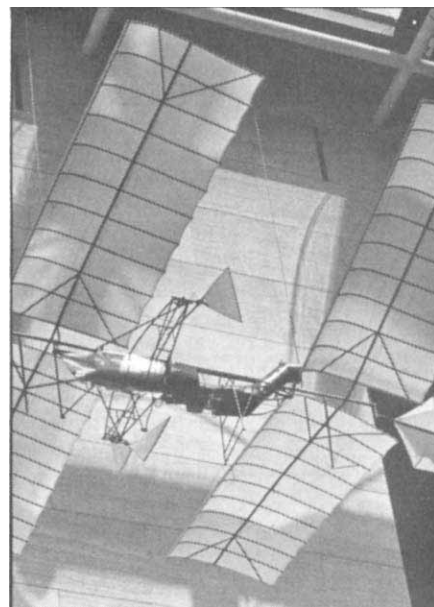
But in the areas where the greatest human transformation has been wrought—in population mobility, in the way people think of time and space and, above all, in the way production is organised—the museum shows its greatest weaknesses. How to remedy this? Perhaps the answer lies in the spacearium and theatre, which currently come closest to surmounting the difficulty with the presentations "Cosmic Awakening" and "To Fly", both pitched to a mass audience, and a mass American audience at that.

Above the two floors of display, together with the staff offices, is the library, available by appointment for any researcher to use. It has provided the main documentation for the show below. Collins says he has sought neither to editorialise nor to impose too much uniformity through excessive analysis. He favours an element of surprise instead, treating some subjects more seriously and some more whimsically than others. He agrees that the aim must be to make a presentation that allows an audience to draw its own conclusions, rather than one which makes particular preconceptions and makes certain conclusions inescapable. He acknowledges that some things need to be dressed up just to catch attention, but would hope that the inevitable built-in bias of any museum is in this case acceptable.

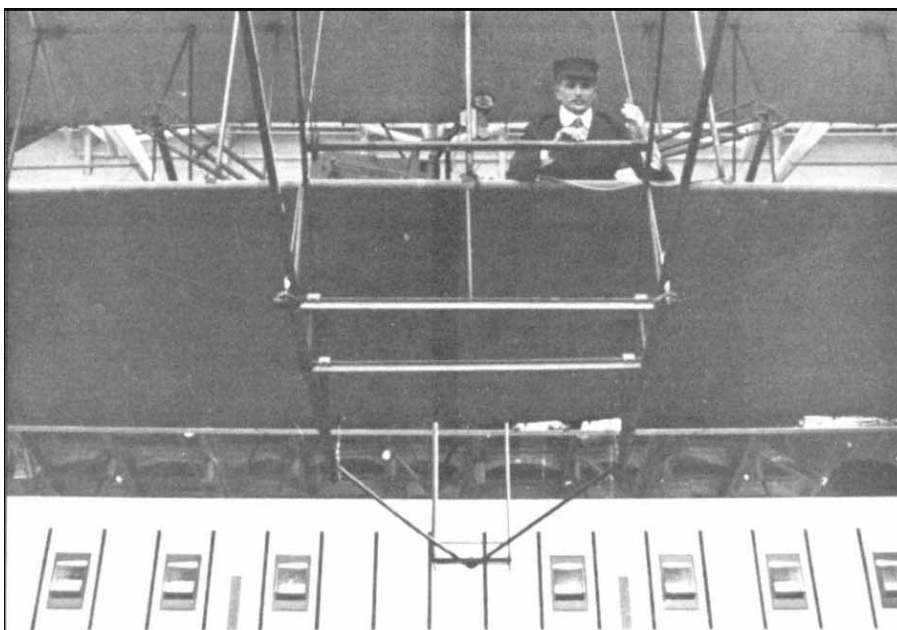
Apparently, it is: if the anti-space or anti-business lobbies in the United States believe that he has in fact failed in his task of educating a curious public, their views have gone largely unrecorded. Collins and his staff are pleased with the press they have received. The only serious regret about such a venture must be that it is a National Air and Space Museum, and not an international one. □



Hall of Air Transportation



Steam driven aerodrome



The Wright Brothers' original Kitty Hawk Flyer

ESA airs itself

Last week, the European Space Agency displayed itself at the Farnborough Air Show in Britain. **Allan Piper** reports

IN solid commercial terms, this year's Farnborough International Air Show brought relatively little joy to the world's aerospace industries, with firm orders for hardware considerably down on recent years. To the many large corporations affected—companies such as Rolls-Royce and Westland from Britain, Aerospatiale and VFW-Fokker from Europe, and Boeing, Lockheed and McDonnell Douglas from the US—the cutback is no less unwelcome for all that it was expected and is seen as only temporary. But few of the contractors present will regard the latest Farnborough as a complete failure. In the first place, the long-term future of the aerospace industry seems secure enough, and this year's record crowds again proved the show's worth as a massive public relations exercise. Second, and far more important, the get-together encouraged a measure of serious discussion on what has become a vital issue for world aviation—international collaboration.

With the enormous expense now involved in the design and development of most civil and military aviation hardware, it is scarcely surprising that collaboration emerged very early during this year's show as the new watchword for aerospace. In Britain, attention focused particularly sharply on planned Anglo-US discussions announced by Gerald Kaufman, the UK Industry Minister.

But for the 12-nation European Space Agency (ESA), prominently represented at Farnborough, collaboration is an already well-established principle. Since its launching almost 16 months ago ESA has put no less than eight scientific satellites successfully into orbit, working with three giant consortia involving many of Europe's aerospace contractors.

While that prolific record itself speaks volumes for the success of European cooperation, early discussions on joint ESA-NASA ventures have ensured that broader collaboration will not go by the board either. The Spacelab project, already under way, represents ESA's largest scientific contribution to the NASA space shuttle programme, planned for the late 1970s and 1980s. Next month, ESA officials are expected to approve funds for another joint project with NASA, the Large Space Telescope. And many of the other new projects in the ESA pipeline will play important roles in forthcoming international programmes.

Meanwhile, Farnborough provided

ESA with an ideal opportunity to stamp its presence on the public's mind. Operating comfortably on a current annual budget of \$600 million, the agency has much to offer in the way of eye-catching space technology. Triumphs have already been notched up with the ESRO, HEOS and COS B satellite programmes, and ESA has an equally impressive timetable drawn up for the next four years or so. New ESA projects include:

- **OTS**, the Orbital Test Satellite, scheduled for launching in mid-1977. Primarily a demonstration model for the European Communications Satellites (ECS) programme, OTS is nonetheless adaptable for various missions. The basic service module can, for example, carry weather forecasting equipment just as effectively as a telecommunications payload. The first operational ECS's will provide around 5,000 telephone circuits and two wide-band television channels along with data transmission and teleconferencing services. During Farnborough a £900,000 design contract for ECS-1 was placed with MESH, one of the European consortia.

- **METEOSAT**, a long range weather forecasting satellite, scheduled for launching next year. METEOSAT will provide Europe's contribution to the Global Atmospheric Research Programme and the World Meteorological Organisation's later World Weather Watch. It will also fit into a global coverage involving Japan, the Soviet Union and the US.

- **GEOS**, the world's first scientific geostationary satellite, will monitor the magnetosphere. Scheduled for launching next year, it will play a major role in the International Magnetospheric Programme.

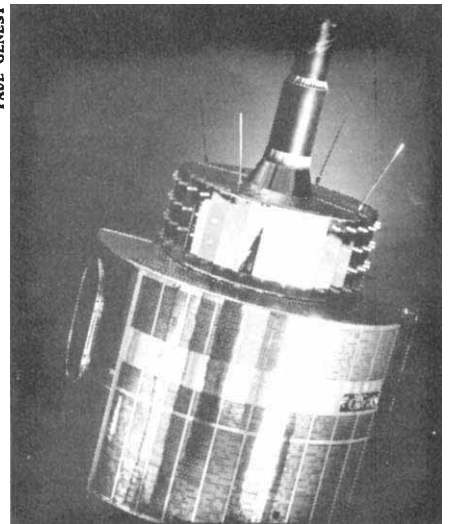
- **IUE**, the International Ultraviolet Explorer, a joint venture between NASA, the UK Science Research Council, and ESA, to be launched next year. ESA will contribute the solar-cell array for the satellite, and will design, build and operate the ground station near Madrid in Spain.

- **ISEE-B**, the International Sun-Earth Explorer, part of another NASA-ESA collaborative project. ESA's ISEE-B will be launched in tandem with NASA's ISEE-A next autumn.

- **MAROTS**, a shipping communications satellite scheduled for launching early in 1978. The payload will sit on an OTS service module.

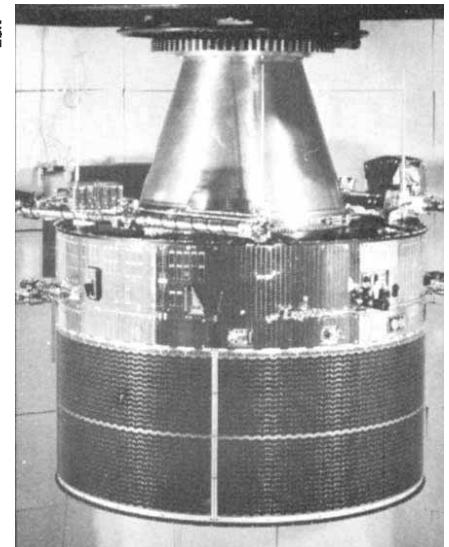
- **AEROSAT**, an aeronautical satellite for ground to air communications on

PAUL GENEST



METEOSAT

ESA



GEOS

Earth. Two AEROSATs will be launched into geostationary orbit at the end of 1979 and beginning of 1980.

- **EXOSAT**, an X-ray astronomy satellite that will monitor cosmic X-ray sources. Along with GEOS, IUE and ISEE-B, EXOSAT will be a purely scientific spacecraft. It will be launched in 1980.

- **Spacelab**, the star of them all. A re-usable pressurised space laboratory, Spacelab will house scientists and engineers working in shirtsleeve comfort. A major component of NASA's space shuttle programme, it will become operational during the 1980s.

- Increasing congestion in the international queue for NASA's launching facilities, which is likely to become worse as NASA steps up its own requirements, has led ESA to develop a heavy launcher, Ariane, for itself. Designed to lift satellites weighing around 800 kg, it could prove crucial to the ECS programme. Ariane's four qualification shots, planned for 1979 and 1980, will be from the permanent launching base in French Guiana. □

USA

Approaching scientific issues

While the Ford administration has been setting up the White House Office of Science and Technology Policy (see page 184), Jimmy Carter's campaign organisation has been establishing links with prominent members of the scientific community. Colin Norman reports

THE campaign between Gerald Ford and Jimmy Carter for President of the United States officially kicked off last week with a round of speeches from the two candidates and their Vice-Presidential choices. Nobody said anything about science, but then nobody really expects scientific issues to play much of a role in this campaign. "Science", as one adviser to the Carter campaign pointed out, "is not exactly the most burning issue in the public mind". Moreover, there's little discernible difference between Republican and Democratic policies for research and development.

But that doesn't mean scientists will play no part in the campaign. Far from it. In fact, it has become something of a tradition for large number of scientists and engineers to shed their usual non-partisan roles and enter the fray on behalf of one candidate or another, and this year will be no different. An extra facet in this campaign, however, is that the Carter organisation has been actively seeking advice from a diverse array of scientists, and that advice has been flowing in quite freely.

It is coming from a very loosely knit science policy task force, whose activities are coordinated by Lewis Branscomb, chief scientist for IBM, former director of the National Bureau of Standards and a well respected figure in science policy circles. Other members of the task force include such equally prominent figures as Harold Brown, President of California Institute of Technology, Harvey Brooks, Professor of Technology and Public Policy at Harvard, David Baltimore, a Nobel Prizewinner from MIT, and George Low, President of Rensselaer Polytechnic Institute.

The science policy task force mirrors similar units advising Carter on a vast range of other issues. It is a very informal, *ad hoc* arrangement involving members who rarely, if ever meet. Instead, they draft policy papers which are sent to the Carter campaign headquarters, to be used as Carter's staff sees fit in drawing up position statements and speeches. As one task force member noted last week, "we haven't exactly got much power in formulating

the policies". This conforms with Carter's well-established approach of seeking opinions from a diverse array of individuals before enunciating his own positions. In foreign policy matters, for example, his advisers include people whose perspectives differ so widely that they must be offering all sorts of conflicting advice which the Carter campaign organisers are filtering and stitching together.

Branscomb said last week that he first became acquainted with Carter late last year, and that he was "impressed with (Carter's) intelligence and approach to scientific issues". Branscomb said that he personally favoured Carter's nomination as the Democratic candidate, and agreed to head the science policy task force when Carter approached him. A measure of the generally non-partisan nature of science policy here is that Branscomb was also a member of a committee advising the Ford administration on an agenda for the new White House Office of Science and Technology Policy (OSTP).

Branscomb has repeatedly emphasised the informal nature of the task force, and its broad membership. Many people, he said, are simply being asked for their views on issues, and their involvement carries no implication that they would necessarily endorse Carter's candidacy. "We are trying to make a contribution to good government," he told *Nature*, and added that the task force is "trying to avoid being an elitist, exclusive club with an inside track".

With Carter already commanding a formidable lead in the public opinion polls, it's clear that the task force is looking beyond November, attempting to lay the groundwork for the first few months of a Carter Administration. In that regard, there has been some speculation that if Carter is elected he will ask Branscomb to be his science adviser and the Director of OSTP. Asked last week whether he would accept such an offer if it is made, Branscomb said the possibility has never been discussed with the Carter organisation, and that he prefers to cross that bridge if he comes to it.

In spite of all the advice flowing into the Carter camp on science policy, so far there is little major difference between the two parties on scientific matters. As far as the party platforms are concerned, for example, though the Republicans devote a few paragraphs to science and technology, the wording is not at all controversial, and could easily have been part of the Democratic manifesto.



Ford, disastrous



Carter, looking forward

The Republican document states, for example, that "every aspect of our domestic economy and well-being, our international competitive position, and national security is related to our past and present leadership in basic and applied research and the development of our technology. But there can be no complacency about our continued commitment to maintain this leadership position". The Democratic platform simply makes no reference to such matters.

Some differences are, however, beginning to emerge between the two candidates on energy and environmental matters, which have some bearing on research and development.

In a speech before the National Press Club last year, for example, Carter attacked "President Ford's disastrous energy policy", which he described as the "Ford/oil industry energy policy", and claimed that it would eventually lead to higher energy

prices and greater reliance on oil imports. He said he was looking forward to the opportunity to formulate his own energy policy, in an atmosphere devoid of crisis. Though he hasn't been too specific about his proposals, Carter has since suggested several times that he wants to see more emphasis on conservation, more spending on solar energy, and more reliance on coal. He has also attacked the Ford Administration's plans to push development of a synthetic fuels industry.

The chief difference between the

Carter and Ford energy policies lies in the area of nuclear power. In a statement made to a United Nations conference last May, Carter suggested that dependence on nuclear power should be held to the minimum necessary, and he has said that he would reassess the priority being given to the liquid metal fast breeder reactor—the most expensive single energy research and development project supported by the Ford administration. He has, however, stopped short of calling for a moratorium on nuclear power, and he

refused to support the California initiative calling for a virtual halt in the development of nuclear power there. (The initiative was defeated in a state-wide referendum last June.)

As election day approaches, science and technology are unlikely to figure very prominently, simply because they do not figure very prominently among the issues about which people are concerned.

If that is a source of encouragement for scientists, it is largely because of the prestige they enjoy. □

AFTER a series of unexpected and often bizarre delays, the newly-recreated White House Office of Science and Technology Policy (OSTP) is finally getting down to business, four months after it was legislated into existence and—if the opinion polls are to be believed—only a few weeks before the Ford administration is scheduled to make its political exit. Even though the administration's days may be numbered in double figures, however, OSTP is being established as if its working arrangements will endure well beyond next January's Presidential inauguration.

H. Guyford Stever, who heads the office and who also holds the title of Science Adviser to the President, said in an interview last week, "I'm not expecting a change of administration. And neither is the President". Accordingly, he is busy recruiting staff, hiring consultants and establishing committees, which means that Jimmy Carter, if he is elected, will inherit a functioning science policy office and some top-level committees whose members would be difficult to replace.

Although legislation establishing the office was signed into law by President Ford on May 11, after taking more than a year to pass through Congress, the OSTP's debut was delayed by an unexpected dispute over the appointment of a director. It had long been rumoured that Ford would nominate Simon Ramo, head of the giant aerospace and defence consortium TRW, as his science adviser and director of OSTP. But the nomination was never made, apparently because Senator Kennedy's office insisted that Ramo would have to divest his holdings in TRW before the Senate would confirm his appointment. Ramo decided that, since the polls all pointed to a resounding Ford defeat in November, it wasn't worth the price to be a science adviser for only a few months. Attention then turned to Guy Stever.

An aerospace engineer and former President of Carnegie-Mellon University, Stever has been Director of

the National Science Foundation (NSF) since 1972 and part-time White House Science Adviser since mid-1973, when Mr Nixon scrapped the old Office of Science and Technology and consigned some of its functions to the NSF. Stever would therefore seem a logical choice for the full-time post of Presidential Science Adviser. But, when news of his impending nomination filtered out, four right-wing Republican Senators warned Ford that they would oppose the appointment. Led by Senator Jesse

weeks. Then, when Ford had the nomination virtually locked up, Stever's nomination was sent to the Senate early in August and swiftly approved with only token opposition.

Stever said last week that he agreed to take on the assignment with the understanding that he wouldn't remain long after January, no matter which administration takes office.

The legislation establishing OSTP also sets up a top-level advisory committee, consisting of between 8 and 14 people drawn from a variety of fields, which will conduct a two-year investigation of federal science support and priorities. One of Stever's first acts was to recommend that Ramo be appointed chairman of that committee, a suggestion which Ford promptly accepted. The other members will be appointed later this month. (Ramo's appointment, incidentally, will present an interesting situation if Carter becomes President, for Ramo was a co-chairman in 1972 of a committee of scientists and engineers supporting the candidacy of Richard Nixon.)

Stever has begun to staff the office with people drawn from NSF's science policy office and a few individuals on secondment from other government agencies. In addition, he has appointed two senior policy consultants, William Nierenberg, Director of the Scripps Institution of Oceanography, and Donald Kennedy, Chairman of the Program in Human Biology at Stanford University. Both will devote half their time to OSTP, assisting in the development of policy studies on biological and environmental issues. Other consultants, Stever indicated, will be called upon as needed.

Asked last week what he considers as OSTP's chief goal, Stever said, "some scientists think that the goal should be to increase the support for science, but they are mistaken. The important thing is to establish an easy relationship at the top level of our government for the scientific community to have an input" into national policy. **Colin Norman**

Man at the top



H. Guyford Stever

Helms, they issued a statement criticising Stever's stewardship of the NSF, resurrecting many of the disputes which have swirled around the NSF's education programmes for the past year.

The opposition surfaced while Ford was locked in his neck-and-neck with Ronald Reagan for the Republican nomination. Stever, mindful of the fact that Ford was trying to steer clear of fights with the party's right wing, suggested that his nomination be reconsidered and the matter was put on the back burner for nine

USSR

● The return of the Salyut 5 crew without having set a new endurance record has aroused speculation abroad as to whether a premature return was necessitated by the psychological stresses of the voyage. This is doubtless due to the fact that it is the first time that the Soviet press has been so explicit about the monitoring of the cosmonauts, which, it appears, involved the analysis of all routine communications with ground control to detect signs of stress.

Cosmonauts Volynov and Zhlobov wanted to be kept up-to-date with world news and sporting events as well as enjoy the on-board entertainments of taped music and a set of photographic slides. In the early part of the flight it was noted that "every detail about the progress of the Olympic Games gave them additional reserves of energy", and during the flight certain innovations were introduced—routine transmission from base were given a background of soft music and special emphasis was placed on "warmth and lively human interest in the concerns of the crew" shown by the communicators.

The official reports, however, do not suggest that the stresses developed were inadmissibly great; the new measures suggest an experiment in remote-control psychotherapy rather than a psychological emergency. Soviet space psychologists have long been concerned with the problems of in-flight sensory deprivation (including the lack of wide human contacts). Over the years this has led to a number of diverse selection procedures for would-be cosmonauts, ranging from the idea that candidates who report monochrome dreams are preferable to those who dream in colour, to the recent pronouncement that no more women will be accepted as cosmonaut-pilots, although they will still be eligible to serve aboard space-craft as "experts"—doctors, astronomers or stewardesses.

● A recent decree of the Central Committee of the Communist Party and the Council of Ministers of the USSR focused on "urgent measures for ensuring for the national economy fuel, electrical and thermal power, and the economic use of fuel and energy reserves". Addressed to all strata of society from the Councils of Ministers of the Union Republics down to the managers of individual enterprises, it called not only for all possible savings but also for the increased use of "peat, shale, briquettes and timber". It was somewhat reminiscent of a speech by Academician Vladimir A. Kirillin, Chairman of the State Committee for Science and

Technology, during his visit to Britain in 1974. This extolled Soviet advances in the technology of fast breeder nuclear reactors, and also stressed that in the immediate future the Soviet power industry envisaged a considerable expansion of open-cast mining for low-grade coal.

The new emphasis on saving and on the use of low-grade fuels may be associated with recent hints of setbacks in the nuclear power plans. Writing in *Pravda*, Academician Nikolai A. Dollezhal (the chief



designer of the reactor for the first Soviet nuclear power station) indicates that there are still considerable engineering problems involved in the construction of nuclear stations, that the manpower involved in installing the equipment is some 2-3 times greater than for thermal stations, and that the efficiency of nuclear stations is generally less than that for thermal.

Moreover, he says, "there are grounds for assuming that in the not too distant future we shall be close to exhausting the 'ecological capacity' of the region of present siting of nuclear power stations." The new economy drive is aimed at ensuring the "unconditional" fulfilment of the five year plan; consequently neither industrial production nor fuel exports will be affected by the measures.

● '*Melioratsiya*', the ten-year-old land-improvement scheme involving drainage and irrigation projects and the general management of soil resources, has received a fresh emphasis. Following the disclosure of new plans over the past few months, *Pravda* has identified the scheme as "a most important task of the whole party, the whole Soviet nation".

This latest move to boost agricultural production includes the resurrection of far-reaching plans to divert northward flowing Siberian rivers south into central Asia and Kazakhstan. In the shorter term, the new measures include calls for substantial increases in the yields of cereal and other crops from "improved lands".

The renewed importance accorded

to *melioratsiya* places involved workers high in the industrial hierarchy: incentives range from a special "feast day" for all concerned, to the title "Master of Irrigation" (First or Second Class) for outstanding individual contributions.

During the recent *Biosfera-76* exhibition in Minsk it was revealed more than 2 million hectares of marshland have already been drained in the Byelorussian SSR; and the past five years have seen an increase in the crop-yield of the reclaimed lands. Nevertheless, erosion is causing losses of up to 20% in crop production, and it is now proposed that some 75% of the peaty soils should be put under grass, leaving only 25% for cereal crops. With the continuing loss of productive soil to industry, and the extensive pollution of water resources also causing serious concern, the indications are that the new plans for *melioratsiya* will require considerable efforts if targets are to be met.

● The Soviet Union has agreed to cease whaling within the next two years, according to a recent statement attributed to Mr Nikolai Makarov, Chargé d'Affaires at the Soviet Embassy in Ottawa. This is a reversal of a long-standing Soviet policy, which maintained that a whaling moratorium would be positively harmful, since it would put an end to research on the biology of whales.

According to a spokesman of the USSR Ministry of Fisheries shortly before the 1974 Session of the International Whaling Commission, the established seasonal quota is insignificant, and there is "no biological necessity" to stop whaling completely since the USSR (which, together with Japan, takes 90% of the world's catch) "consistently and accurately observes all the provisions of the Whaling Convention, while attaching specially great importance to the development and implementation of effective measures to protect the whales".

At the 1976 session in June, the Soviet Union strenuously opposed the proposal to set quotas by "yield by weight", rather than by the "maximum sustainable yield" introduced in 1961. The USSR disapproved of the change, to be applied in the first instance to sperm whales only, arguing that the Commission had already done much in the past two years to maintain sperm whale stocks.

The reversal of policy is more than a cause for satisfaction among conservationists; hitherto, it is said, sperm whale oil has played an important role in the manufacture of Soviet tanks and missiles. **Vera Rich**

IN BRIEF

Williams the DES

In a Cabinet reshuffle Shirley Williams last week took over the post of UK Secretary of State for Education and Science from Fred Mulley. While Mr. Mulley's low-profile tenure of the post—marked by a period of economic stringency, cuts and out-of-work teachers—led to little in the way of long-term science policy, hopes are high among scientists and policymakers that Mrs. Williams will provide a different style of leadership, taking science under her own wing (rather than leaving it to a junior Minister) and generally infusing some optimism into what is at present a gloomy scene.

Mrs. Williams was a junior Minister for Education and Science between 1967 and 1969; she returns to the Department from her position as Secretary of State for Prices and Consumer Protection, held for the past two years.

More for US research

Government spending on science and technology in the United States is expected to reach \$23,500 million during the fiscal year beginning October 1, according to figures recently published by the National Science Foundation (NSF). That would represent a modest increase from this year's level of support, even when inflation is taken into account.

The welcome increase follows a long period of decline in the federal science budget, as inflation has gradually eroded its purchasing power. The increase will be sufficient only to restore science support to its 1972 level, however, which in turn was 20% below that of 1967, the peak year of federal science support. The growth next year is almost solely attributable to large increases in funding for energy and defence research and development.

New orchid for Britain

Whatever its effects on wildlife in general, this year's warm dry spring brought a notable addition to the British flora: the orchid *Ophrys bertolonii*. A single specimen has been found near Swanage, Dorset, growing in undisturbed downland turf; how it got there will remain a mystery, unless some enthusiast confesses to having planted it there as a seedling some years ago. One of the loveliest and most unmistakable of European orchids *O. bertolonii* is essentially a plant of the western Mediterranean, although it occurs north of the Apennines and is also recorded from Bulgaria.

The notion that the British specimen has been in position for some years without flowering is strengthened by the discovery in Gloucestershire, also this season, of a second species, *O. sphegodes*.

It must have come as a surprise to most people in Britain to learn that, according to the statistics for food consumption recently published by the UK Ministry of Agriculture, Fisheries and Food (MAFF), the nation's population is on average eating less than the minimum diet recommended by the United Nations Food and Agriculture Organisation (FAO). Britons are apparently saved from starvation only by the efforts of the confectionery and brewing industries, whose products contain sugar that makes up for what would otherwise be a significant deficiency in calories.

It is difficult to take these figures seriously. If the average level of consumption is so near the starvation line, then something like half of the UK's population should show symptoms of undernourishment, for the least-privileged members of society probably eat fewest sweets and drink least beer. Yet it is rare to see anyone except a criminally neglected child or an uncared-for elderly recluse, who is seriously underweight. One cannot walk along a city street in Britain without seeing many who are grossly overweight. The school medical service complains about the increase in the number of obese children. Surveys show that as many as 75% of middle-class and middle-aged women, and over 50% of men, worry because they are overweight. Food advertisements push products that are low, not high, in calories. Newspapers are full of diets devised to allow consumers to lose weight without suffering the pangs of hunger. As a nation Britain shows all the signs of overeating, not of consuming

too little. The MAFF not only suggest that Britons eat too little food of any kind, but also that "the quality of food consumed shows a steady decline". This view is based mainly on data showing falls in the consumption of meat and other animal products, and of sugar. But as Dr. J. V. Durnin,

MAFF gaff



KENNETH MELLANBY

a nutritionist at Glasgow University, has commented in *The Times* newspaper, these changes should be viewed as a limited improvement, and not a deterioration in feeding habits. Moreover, they accord with a recommendation from the Royal College of Physicians.

The MAFF seems intent on perpetuating the fallacy that, because some protein is necessary for health, the consumption of more animal

products leads to better nourishment. Some time ago, the Minister even suggested that any reduction in the amount of meat eaten in Britain would give rise to serious malnutrition. Britain's diet may indeed be changing. Rising costs may be preventing everyone from consuming all the things they might wish to eat. Some people may be getting less pleasure from eating, or, more likely, from overeating. But in presenting dietary data, the MAFF should surely pay heed to the principles of nutrition, rather than to those of hedonism.

Those living in Britain can observe the real situation, but what will observers in other countries, or in organisations like the FAO, make of the MAFF statistics? Will the UK's 55 million citizens be added to the thousands of millions in countries even poorer than Britain already listed as suffering from food shortages? In the past, many statistics published by international bodies have exaggerated world starvation and malnutrition. It is revealing to find that equally exaggerated data can be produced for a country like Britain, where accurate information might be expected.

Yet there are too many hungry and even starving people in the world today. There are many countries where overeating is not, as it is in Britain, the main cause of 'malnutrition'. The problem can only be solved if there are accurate statistics for food consumption, and proper estimates of food requirements, for all countries. A start should be made by producing such information for Britain.

correspondence

Ball lightning

SIR,—Ball lightning is a relatively rare phenomenon, usually occurring under conditions of very strong electrical fields and usually associated with storms, though not necessarily in the storm area. The balls can be from one inch to several feet in diameter and can have various colours. Some explode violently and some fizzle out quietly. The balls move through the air, apparently independent of wind and gravity, and are known to enter structures—even through a metal window screen and into a metal aircraft. There is no good theory to explain ball lightning, but J. R. Powell and D. Finkelstein are working on a d.c. field theory in which a strong electric field adds energy to the ball to sustain it (*Structure of Ball Lightning*, Brookhaven National Laboratory, Upton, New York).

Little is known about the ignition hazard of ball lightning, but in the course of investigating accidents in the past ten years, we have heard the following statements from witnesses:

- Distillate was being loaded into the open top of an 8,000 gallon transport truck. "A ball of light travelled along the fill pipe and down the droptube. It entered the truck compartment and the truck blew up."

- Solvent was being loaded through closed piping into the bottom of a 7,600 gallon transport truck. "A ball of light followed along the fill pipe toward the truck and disappeared. Then the truck blew up."

- An observer on a ship several miles from an empty VLCC (very large crude carrier) tanker. "A ball of light travelled along the deck of the ship and disappeared. Then the ship blew up."

- Jet fuel was being loaded into the open top of a transport truck. "A ball of light entered the truck compartment and it blew up."

- A retired New York City fireboat captain, Charles Wilson of Codan Marine Inc., New York. "I know of three cases where a ball of light was reported to have moved across the deck of a barge just before the barge blew up."

- A tanker captain with the same corporation, Captain Wykoff, reportedly "saw a ball of light move across the deck toward a compartment before it blew up."

Taken individually, these are each

incredible and are usually ignored by safety experts. Since there are these eight cases reported by separate observers, it appears that ball lightning might be an infrequent and unrecognised source of ignition.

There probably are other incidents, and we are soliciting any information that others may have. Our concern is that this is an unrecognised source of ignition in fuel transport operations and that conventional protective methods may be inadequate.

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Forecasting error

SIR,—Kenneth Mellanby, in a short but cogent article ("Forecasting Error", August 5, page 441), is highly critical of the state of the art of forecasting many of the social and economic components of present society.

Most of his strictures are only too true. I would however take issue with his implication that "only some form of rigid totalitarianism could improve the statistics". This can surely be tested by examining the forecasting skills of societies which have for some time displayed this characteristic. To my knowledge there is no evidence that they do have a greater predictive success rate; in fact the impression is quite the reverse.

To improve data sources used in predictions would involve the public as individuals and groups in providing information on present and intended actions and aspirations which, for various reasons, they are unwilling to divulge with any degree of spontaneity. At least part of this unwillingness stems from a sense of isolation from the decision-taking which would (hopefully) be based on the information. If truly participatory decision-taking processes existed, after a due period of time to allow for cultural inertia, the inclination to provide adequate information from which to derive alternative policy plans might well emerge.

Better data require more democracy, not less.

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Mistaken parameter

SIR,—Politicians and others have been criticised in the national press for the misuse of the word "parameters", but I have yet to see similar charges levelled against scientists who have much less excuse for this practice. During the past three years the tendency to describe components, factors, attributes and so on as parameters has become frequent in most scientific journals, covering agricultural research and related fields, published in the UK and abroad.

To anyone in the habit of using parameters with gay abandon I would recommend the definition given by Professor D. J. Finney in *An Introduction to Statistical Science in Agriculture*:

"Numerical values that serve . . . to identify a particular distribution curve, as a member of a whole family, are termed *parameters* . . ."

I would predict that if the term were restricted to numerical values, without any greater precision of meaning, the frequency with which it is used would be halved.

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Electrifying demonstration

SIR,—The theoretical approach by Marble, MacVicar and Roberts (Correspondence, July 15, page 171) is not necessary. For many years, when teaching electrostatics to first year students, I used to stand on a 5 cm thick block of paraffin wax and charge myself up from a small Van de Graaf generator capable of giving 300,000 volts on open circuit. This made my hair stand on end in a convincing manner, and the sparks which I could get from a hand-held object to earth would certainly have ignited any explosive gas mixture.

Without wishing to compete with the above authors in numbers of significant figures, I must have reached between 100,000 to 200,000 volts with respect to Earth without harm—incidentally illustrating for the sake of the students the old GEC adage: "It's the amps, not the volts, wot kills yer".

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news and views

Eukaryotic mRNA: trouble at the 5'-end

from Beverly Griffin

UNTIL recently, the story of the 5'-termini of eukaryotic messenger RNAs seemed consistent and scientifically coherent. The evidence emerging seemed to confirm two separate conclusions. First, eukaryotic messenger RNAs contain 7-methylguanosine triphosphate, $m^7G(5')ppp(5')X$, at their 5'-ends. To use the terminology introduced by Rottman *et al.*, they are "capped".

This seemed to hold true for mRNAs from a large number of cells: mouse myeloma cells² and L cells^{3,4}, HeLa cells⁵⁻⁷, Novikoff hepatoma cells⁸, Ehrlich ascites carcinoma cells⁹, brine shrimp (*Artemisia salina*) cells¹⁰ and silk fibroin (*Bombyx mori*) cells¹¹.

The same 5'-terminal ends were found for mRNAs from both DNA and RNA viruses, such as: reovirus¹², Newcastle disease virus¹³, vaccinia virus^{14,15}, Sindbis virus^{16,17}, avian sarcoma (ASV) virus^{18,19}, vesicular stomatitis virus^{20,23}, adenovirus²⁴, and cytoplasmic polyhedrosis virus²⁵. They were also found in several plant viruses: tobacco mosaic (TMV) virus^{26,27}, alfalfa mosaic virus²⁸, and brome mosaic virus²⁹.

Translation requirement

Second, the presence of the 5'-terminal 7-methylguanosine (that is, the "cap") is required for translation. Two separate, but related, studies using mRNAs from reovirus or VSV showed that the removal of the cap resulted in a loss of translational activity in *in vitro* protein synthesising systems^{30,31}.

Following on these findings, other results on the how and when and where began to emerge. Aurin tricarboxylic acid (ATA) which inhibits translation, was found by Both *et al.*³¹ also to inhibit messenger RNA methylation, leading them to suggest that the cap structure is important in messenger-ribosome complex formation, the absence of 7-methylguanosine markedly reducing formation of the complex. Rose²² showed that messenger RNAs with 5'-triphosphate ends, but no 7-methylguanosine, were present in VSV but were not found on ribosomes;

he therefore postulated that capping might have a role in ribosome recognition of mRNA. Further corroboration and refinement came from the results of Roman *et al.*³², who, studying mRNA binding in a fractionated wheat germ system, obtained results which suggested that the capped mRNA was essential for the interaction of the 40S ribosomal subunit with the 60S subunit to give the 80S ribosomal complex; in competitive inhibition studies, 7-methylguanosine-5' phosphate was found to have no effect on the formation of the 40S complex, but to inhibit the transition of the 40S ribosome-met-tRNA^{Met} complex to the 80S ribosome complex. Similar experiments were carried out in other laboratories and similar results obtained³³⁻³⁵. In some interesting studies with model compounds, Both *et al.*³⁵ found that the presence of 7-methylguanosine triphosphate at the 5'-end of some ribopolymers was not in itself sufficient to ensure ribosomal binding. For example, capped poly-riboadenylic acid did not bind to any significant extent, whereas capped poly-ribouridylic acid could form 40S complexes, and capped ribopolymers containing both A and U could form 80S complexes. Their results strongly implicated not only the 7-methylguanosine in ribosome binding, but also pointed to the need for sequences rich in A and U for stable binding. It does seem important to note here that some ribopolymers such as (A,U)_n bound stably to ribosomes (even though they did not contain a 7-methylguanosine cap). The dependence on the presence of adenosines and uridines for stable complex formation between eukaryotic mRNA and ribosomes makes the sequence of brome mosaic viral RNA fragment protected by eukaryotic ribosomes all the more interesting; Dasgupta *et al.*²⁹ found the sequence which precedes the translation start signal in this message to be $m^7G(5')ppp(5')GUAUUAUA$. . . , a sequence notably rich in A and U.

For a further probe into the role of capped mRNAs in protein synthesis, Shafritz *et al.*³⁶ studied the interaction

of mRNAs from VSV, EMC, and histone mRNA with purified initiation factors. They demonstrated complex formation between mRNAs and factors IF-MP, IF-M3, and IF-M2A (but none with IF-M2B). For inhibition studies, they used excess 7-methylguanosine triphosphate, and in only one case, that with IF-M3, was this nucleotide able to inhibit messenger-factor complex formation. Their conclusion from their studies was that initiation factor IF-M3 might be essential for the binding of certain mRNAs to ribosomes.

Exceptions

So far, so good. And now the trouble starts, and we come to the findings that begin to question the validity of the above observations as generalisations to all eukaryotic mRNAs. Two papers which appeared simultaneously say that polio viral mRNA is not capped by its 5'-terminal's being pUp^{37,38}. Moreover, encephalomyocarditis (EMC) virus mRNA appears to contain no capped 5'-end³⁹, nor do the mRNAs from the plant virus, satellite tobacco necrosis (STNV) virus^{33,40}. So not all eukaryotic mRNAs are capped.

Moreover, a paper has now appeared which says that translation does not require 7-methylguanosine; Rose and Lodish⁴¹ were able to translate mRNAs of VSV in an *in vitro* system even after the terminal 7-methylguanosine had been removed. Thus, translation of some messengers in some protein synthesising systems may not require the terminal cap.

To add further to the uncertainties, Filipowicz *et al.*⁴² find a cap-binding protein in extracts of brine shrimp embryos, but this protein which binds to mRNAs and is postulated to promote binding to ribosomes, is not IF-M3, nor indeed does it appear to be any of the other protein initiation factors.

It is clear from the paradoxes mentioned above, that different as well as better-defined experiments need to be carried out in order to reach definite conclusions about the structure and

function(s) of the 5'-end of eukaryotic mRNAs. With few exceptions, most of the studies to date have been so similar in kind that if a trap is there, it would have captured in turn almost every investigator. For example, most studies on mRNA have been carried out on species purified *via* the polyadenylated 3'-ends of the RNAs. But not all mRNAs contain poly(A), and who can yet say that poly(A) may not be added merely to "store" mRNAs until they are needed, and then removed to produce "functioning" messages? (Marbaix *et al.*,⁴³ point to the role of poly(A) 3'-terminal sequences as one of stabilising eukaryotic mRNAs.) Moreover, most studies on the function of the cap at the 5'-end of mRNAs have used mixtures of messenger RNAs and the wheat germ *in vitro* translating system. (There are a few exceptions to this criticism. For example, see Yang *et al.*¹¹ and Shafritz *et al.*³⁶ for use of the rabbit reticulocyte cell-free protein synthesising system.) Even after considering the exceptions, it is not clear whether one is justified in drawing firm conclusions about mRNAs from a single protein synthesising system. It seems from the data accumulated so far that studies on mRNAs may merely reflect the idiosyncracies of the individual *in vitro* translation systems. Moreover, with the mRNAs that fail to be translated, no real proof exists that the chemical procedures used to remove the capped ends have not damaged the RNAs in other ways. Rose and Lodish⁴¹ say: "We initially had great difficulty recovering translatable VSV mRNA after periodate-oxidation and β -elimination, and clearly did not completely overcome the problem of degradation. Thus, we suggest that similar studies indicating a requirement for 7-methylguanosine in translation of cellular mRNAs should be more carefully controlled for possible non-specific effects of the chemical procedure".

If 7-methylguanosine survives as a requirement for translation for most mRNAs and in most protein synthesising systems (as well as in the cell?), it will be interesting to see whether mRNAs for which no cap has been found, such as those from poliovirus or EMC, have a 7-methylguanosine (as a modified base) anywhere near their 5'-termini.

More about the 5'-end

What else do we know about the 5'-ends of eukaryotic mRNAs? Most eukaryotic mRNAs, in addition to containing 7-methylguanosine triphosphate at their 5'-ends, also appear to contain 2'-O-methylated bases as the first, and frequently the second, nucleoside following the capped end. Some exceptions to this have been found, notably among the viral mRNAs. (For ex-

Salt on roadside verges

from Peter D. Moore

THE impact of disturbance upon the flora of roadside verges is a subject of considerable interest to conservationists and to environmental planners, for the habitat occupies a large area of our country and provides an opportunity for combining utility with aesthetic and scientific interests. In the main, interest has centred upon such effects as lead pollution from motor exhausts, but an additional stress imposed on these verges is provided by the salt which is applied to roads in winter.

The problem was recognised and discussed by Westing (*Phytopathology*, **59**, 1174; 1969) in America where, at the time of his survey, some 6×10^6 tons of salt were applied to the highways of the northern states during the winter. This figure was increasing by about a million tons every year. In the United States, applications of 6 kg m^{-1} along the side of the road are frequent. In Britain, Davison (*J. appl. Ecol.*, **8**, 555; 1971) estimated that applications of 3 to 4 kg m^{-2} were experienced by the roadside verges of Northumberland.

The presence of salt in the soil lowers the water potential and influences soil structure, which may make water less available and reduce aeration for the resident plants. The effects on trees have caused concern in the United States, where Westing (*Econ. Bot.*, **20**, 196; 1966) has described a decrease in the population of the sugar maple (*Acer saccharum*) caused by a combination of drought and salt applications. Low rainfall in

summer, of course, can result in reduced leaching and the persistence of salt applied during the winter.

In Britain, trees are not permitted to grow close to the edge of roads, so are not subjected to the greatest stress; this is felt instead by the characteristic grasses and herbs. Analysis of verge soils by Davison (*op. cit.*) showed that sodium levels in April could be ten times those in unaffected grassland, but usually fell to normal by September. Sodium concentrations in plant leaves were often five to ten times the normal level.

It may be expected that the continued application of salt to roadsides would result in the selection of more tolerant species and this has now been observed by Matthews and Davison (*Watsonia*, **11**, 146; 1976) in areas around Newcastle-upon-Tyne, Northumberland. In a survey of changes in roadside flora they describe reduced species diversity and the selection of resistant species. They also describe an invasion of maritime species, such as *Aster tripolium*, *Puccinellia maritima*, *Plantago maritima* and *Sueda maritima* up to 13 km from the sea. These halophytic species have been particularly effective in the colonisation of new (less than 3-yr old) roads. It is likely that these species have been brought in as seeds on the wheels of vehicles. It will be interesting to see whether the development of a saline roadside flora continues along our highways in a similar manner to the establishment of a railway flora in the last century.

ample, see the references to Newcastle disease virus, Sindbis virus and TMV virus mRNAs.) Structures of the type $\text{m}^7\text{G}(5')\text{ppp}(5')\text{X}^{\text{m}}\text{pYp}$ are now being called cap I structures and those of the type $\text{m}^7\text{G}(5')\text{ppp}(5')\text{X}^{\text{m}}\text{Y}^{\text{m}}\text{pZp}$ are being called cap II structures⁴⁴. Perry and Kelley have carried out a very interesting study on the kinetics of methylation of the 5'-ends of mRNAs. Their results suggest that the 5'-terminal cap I structures of mRNAs are derived from capped heterogeneous nuclear RNA and are conserved during processing. Cap II structures, on the other hand, are not found in the cell nucleus, but arise by a secondary methylation after the mRNAs have entered the cytoplasm. This secondary methylation appears to be restricted to a particular subclass of mRNAs which have a high frequency of pyrimidine nucleosides at position Y (see above), and are among the more stable

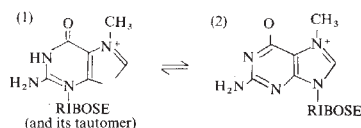
mRNAs. Studying mRNA from cytoplasmic polyhedrosis virus, Shimotohno and Miura⁴⁵ have shown that methylation at the messenger 5'-end occurs first at the 7-position of guanosine, and only after chain elongation has proceeded for a few nucleotides is the 2'-position of the first base which follows the cap structure (adenine, in this case) methylated.

(The discovery in cell extracts of enzymes which cleave at the 5' terminal and remove the cap will no doubt help resolve some of the problems and conflicts now being encountered in studies on eukaryotic mRNAs. Three papers have appeared which report on the presence of such an enzyme activity in HeLa cells⁷, in extracts from brine shrimp⁴⁶, and in cultured tobacco cells⁴⁷.)

One of the early papers in this field, and probably the one which was most influential in bringing the capped ends

of mRNAs to the attention of the scientific public was the paper by Rottman, Shatkin and Perry¹ entitled "Sequences Containing Methylated Nucleotides at the 5' Termini of Messenger RNAs: Possible Implications for Processing". In one of the most important papers to appear and challenge the ideas widely quoted (and therefore beginning to be accepted as dogma) on the function of the cap in eukaryotic mRNAs, Rose and Lodish⁴¹ say that their (conflicting) results "suggest that methylation is involved in synthesis or processing (VSV) mRNAs into the correct poly(A)-containing mRNA species". And so, have we gone through so much only to come full circle?

I should like to conclude by making a few remarks. First, 7-methylguanosine is an extremely interesting molecule. One of the early papers on capped structures referred to the cap as a "bizarre" structure. The better word might be "versatile". Haines *et al.*⁴⁸ found 7-methylguanosine to have a potentiometrically determined pK_a of 7.0. This means that at pH 7.0, it exists as an equilibrium between two ionic forms:



Lower the pH, and form (1) becomes more important. Raise the pH, and form (2) becomes more important. The implications of this are that the capped structure would be expected to be remarkably sensitive to

localised pH changes within the cell.

In an unusually interesting paper in a too-neglected field, Seeman *et al.*⁴⁹ have studied sequence specific recognition of nucleic acids by proteins. For double-helical RNA (or DNA), their analyses lead them to conclude that whereas single hydrogen bonding interactions were inadequate to explain the specific protein-nucleic acid complexes they observed in model studies, pairs of hydrogen bonding interactions might play a role in protein-nucleic acid recognition. To return to the capped structure, within this structure (even ignoring any effects due to phosphate groups) would appear to be the requisite qualifications for specific interactions with proteins; cursory examination would suggest a possible stable interaction with, for example, the guanidinium group of an arginine molecule. With a slight change in pH, the tautomeric form of 7-methylguanosine could change and the complex be thereby strengthened, or weakened, depending on the nature of the change. The word versatile reflects the potential of the capped structure to respond in a very sensitive way to its environment; its environment in turn can confer on it the properties for self-regulation, whether in messenger binding or processing or transport.

The questions raised by the presence (or absence) of the cap at the 5'-ends of eukaryotic mRNAs have clearly stimulated considerable interest, and even that form of excitement which is so important to scientific research. But the tale is unfinished. And so, the reader is left with the opportunity to carry on with the story . . .

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Why do stars twinkle?

from B. J. Uscinski

ALTHOUGH the twinkling of stars is apparently a simple phenomenon, the theory which has been developed to explain it is anything but simple. The article by Jakeman, Pike and Pusey on page 215 of this issue of *Nature*, shows that it is now possible to test the validity of the new multiple-scatter theory which has evolved over the past 10 years. This is an important step forward, since the theory is essential not only for the study of optical scintillation, but also in investigating the scintillation of quasars and pulsars caused by the interplanetary and interstellar media respectively. Up till now it has not been possible to make satisfactory experimental tests of the new body of multiple-scatter theory since detection and recording techniques have not been sensitive enough.

The early 1960s saw the beginning of

serious efforts to study the propagation of waves in media containing random irregularities of refractive index. One of the first attempts to deal with the case when the intensity fluctuations of the wave become large was the so called 'Method of Smooth Perturbations'. (Obukhov, *Izv. Akad. Nauk SSSR, ser. geofiz.*, No. 2, 155; 1953). This method became widely known, having appeared in a book by Tatarsky (*Wave Propagation in a Turbulent Medium* (trans. by R. A. Silverman), McGraw-Hill, New York, 1961), but it was soon realised that it was not valid for the case of multiple scatter (Barabanenkov *et al.*, *Usp. fiz. Nauk*, **102**, 1; 1970). Subsequent methods based on repeated application of the Born approximation were able to deal adequately with the multiple-scattering of waves in media where the perturba-

tion caused by a single irregularity is small (Shishov, *Izv. Vyssh. Ucheb. Zaved. Radiofizika*, **11**, 866; 1968; Tatarsky, *Zh. Eksp. Teor. Fiz.*, **56**, 2106; 1969; Beran and Ho, *J. opt. Soc. Am.*, **59**, 1134; 1969). This came to be known as the Markov approximation, and such media as "weakly irregular" media.

The new theory is based upon a set of partial differential equations for the various moments of the field, and theoreticians generally agree that they are valid in the case of multiple scatter (Barabanenkov, *op. cit.*). The equations have several interesting features. First, the variables can be scaled in such a way that each equation contains only one parameter, which is the same for all the equations (Barabanenkov, *op. cit.*). Hence all weakly irregular media can be classified as regards their scattering properties in terms of a single parameter. Second, the level of intensity fluctuations, sometimes called the "scintillation index"

or the "normalised contrast", found by solving the equation for the fourth moment exhibits peaks or foci where values well in excess of unity occur in certain conditions (Dagkesamanskaya and Shishov, *Izv. Vyssh. Ucheb. Zaved. Radiofizika*, **13**, 16; 1970; Shishov, *Zh. Eksp. Teor. Fiz.*, **61**, 1399; 1971). Finally, a marked reduction in the scintillation index or normalised contrast is predicted when the signal is received over a wide frequency band.

It is indeed interesting that Jakeman *et al.* have been able to observe these last two effects. As the authors point out, earlier failures to observe the predicted effects have probably been due to instrumental insensitivity and averaging of the response. They do not necessarily indicate that the theory is wrong. The new detection techniques can now be put to an obvious and necessary use. Multiple scatter theory can be tested experimentally. This is essential since the theory is widely used as the basis for investigating stellar objects, as well as various aspects of both the interstellar and interplanetary media. It is also used when probing the atmosphere with laser beams.

The tests would have to be carried out in controlled conditions in a laboratory. As Jakeman *et al.* remark, some of the effects to be measured are heavily dependent on the model. If naturally-occurring irregular media are used it is difficult to measure the autocorrelation function and scale size of the irregularities. Moreover, there could well be a mixture of scale sizes present, and the statistics of the medium could be non-stationary. A stationary irregular medium with a single scale size and an autocorrelation function which can be determined could be synthesised in the laboratory from irregular plastic layers for example. In these conditions the new detection techniques would allow the predictions of multiple-scatter theory to be systematically verified, especially the focusing effect and the classification of media in terms of the single parameter.

In addition, the probability distribution of the wavefield could be determined for different scattering regimes. It has sometimes been asserted, with little theoretical justification and no qualification, that the logarithm of the amplitude is normally distributed. There seems to be more reason to suspect that the probability distribution is different for different regimes, and while being similar to a log-normal distribution in some circumstances is more like a Gaussian in others (Barabankov, *op. cit.*).

Until controlled tests of this type are carried out, multiple-scattering theory will remain open to doubt and criticism. □

New limits on variability of fundamental physical quantities

from Paul Davies

ONE of the greatest mysteries of nature is why the various dimensionless ratios of fundamental physical quantities take the apparently arbitrary values that we find for them. Why, for example, should the proton mass M_p be 1836.1 times greater than the electron mass M_e and not some other, equally meaningless, number? Or why should the square of universal electronic charge, e^2 , be $1/137.036$ times the product of Planck's constant $\hbar$ and the speed of light c ? If any old numbers will do, why not something elegant, like 1 or π ?

Three schools of thought have arisen about these ratios. One supposes that the numbers are not arbitrary at all, but will emerge as a precise result from a future theory of matter, in the same way that the anomalous magnetic moment of the electron (0.00116 times the "normal" magnetic moment) may now be derived from quantum electrodynamics. The second does not attempt to explain these numbers, but points out that if they were very different from their observed values, it may not be possible for them to be observed—because the existence of living organisms (to do the observing) depends on many complicated processes more or less sensitive to these values.

The final school of thought is a more radical one, proposing that the numbers actually observed are indeed nothing special, because those numbers are not in fact constant, but are changing with time. Thus, the values 1836.1 or 137.036 for example, are just the 1976 values, and have no fundamental significance.

The reasoning which led to this idea arose in the 1930s from a study of cosmology. Several physicists and astronomers, most notably P. A. M. Dirac and A. S. Eddington, drew attention to the very large dimensionless ratio which one obtains by expressing the age of the universe in atomic units of some sort. This number is about 10^{10} . By apparent coincidence, 10^{10} is roughly the same ratio by which electric forces are stronger than gravitational forces between two atomic particles. Dirac argued that any fundamental theory of matter would be unlikely to throw up a number so preposterously large as 10^{10} so that perhaps the latter ratio is actually determined by the former, that is that there is a causal connection between these two large numbers. If that is so, then as the Universe grows older, and the first ratio increases, so must the force of gravity dwindle in relation to electromagnet-

ism. This could come about by a decrease of Newton's gravitational "constant" G , for example, or by an increase of the electronic charge e .

Fortunately, it is possible to provide observational checks on the hypothesis that some of these ratios may be time-dependent. This is because modern technology enables us to study matter in regions of the Universe which are so distant that the light from them has taken several billions of years to reach us. Therefore, we see those regions as they were when the Universe was only a fraction of its present age. Any variation in the numerical ratios which is based on a cosmological cause might therefore be expected to show up in the spectra from distant galaxies or quasars. The redshift of the spectral lines fixes the distance, and hence the age of the source according to Hubble's law of the expansion of the Universe. But if it is also possible to measure the fine structure of the spectral lines (a small splitting due to relativistic effects in atoms) then a value for the fine structure constant $\alpha = e^2/\hbar c$ may be determined. In 1967 Bahcall and Schmidt were able to fix, using this technique, the possible variation in α to be below about one part in 10^{12} per year, thus ruling out the theory that G is fixed and e^2 increases in proportion to the age of the Universe (about 1 part in 10^{10} per year).

Recently, not only fine structure, but hydrogen hyperfine structure has been detected in a very distant radio source. This hyperfine structure is an electromagnetic effect which depends on the small coupling between the proton and electron in the hydrogen atom, and its measurement yields a value for the ratio $\alpha^2 M_e/M_p$. In a recent issue of *Physical Review Letters*, three American astronomers, A. M. Wolfe, R. L. Brown and M. S. Roberts use the new observation of hydrogen hyperfine absorption to fix the variation of $\alpha^2 M_e/M_p$ to as little as two parts in 10^{14} over at least 35% of the age of the Universe (the "look-back" time of the source). They also confirm Bahcall and Schmidt's result by observing fine-structure in the magnesium spectrum from this source. These results taken together (and assuming the internal structure of the proton does not change) completely rule out any small power variation of α or M_e/M_p over cosmic time scales. Of course, Dirac's theory can only be ruled out by simultaneously providing a restriction on the variation of G , a much more

difficult task.

Although this is a negative result, the importance for theoretical physics of keeping the natural constants constant makes these occasional cosmological checks well worthwhile. In the absence of a theory of natural constants, the significance of their numerology will have to be left to the philosophers to ponder.

Herpes virus induction of F_c receptors

from R. S. Kerbel

WHEN cells are infected with viruses or are transformed by oncogenic viruses, alterations in plasma membrane components often occur. The characteristics of these surface changes and how they may relate to the biological behaviour of the cells have been popular areas of study for both virologists and immunologists alike.

One example of how spectacular these changes can be concerns induction of surface receptors, specifically F_c receptors. Over 12 years ago, Watkins noted that cultured HeLa cells were able to absorb antibody-coated erythrocytes and form rosettes after the cells were infected with herpes simplex type 1 virus (*Nature*, **202**, 1364; 1964). This was later confirmed by Yasuda and Milgrom who used various different cell lines derived from both man and monkey (*Int. Arch. Allergy*, **33**, 151; 1968).

We now know that a variety of cells, mostly lymphoreticular in nature, have surface receptors for the F_c portion of IgG immunoglobulin molecules (F_c receptors). These receptors can be detected by erythrocyte-antibody (EA) rosette formation. Thus, it seems that infection with herpes simplex virus (HSV) results in induction of F_c receptors, a conclusion substantiated by Yasuda and Milgrom who showed that virus-infected cells could not absorb erythrocyte coated with $F(ab')_2$ antibody fragments, but only with the fully intact antibody molecules.

The gene which codes for the receptor seems to be virally coded, and not a repressed host gene switched on by viral infection (Westmoreland and Watkins, *J. gen. Virol.*, **24**, 167; 1974). Subsequent studies have shown that mammalian cells such as hamster embryo fibroblasts oncogenically transformed by inactivated HSV types 1 and 2 can also display F_c receptors (Westmoreland, Watkins and Rapp, *J. gen. Virol.*, **25**, 167; 1974).

It was only natural to ask whether other members of the herpes family of viruses could also induce the appear-

ance of F_c receptors in infected cells and an affirmative answer was recently provided by Furukawa *et al.* who found human fibroblasts displayed F_c receptors after infection with human cytomegalovirus (*J. clin. Microbiol.*, **2**, 332; 1975), an observation also made by Rahman *et al.* (*J. Immun.*, **117**, 253; 1976), Keller *et al.* (*J. Immun.*, **116**, 772; 1976) and Westmoreland, Jeor and Rapp (*J. Immun.*, **116**, 1566; 1976).

Now that every type of cell thus far tested from over six different species has been shown to develop F_c receptor sites after infection by at least two different herpes viruses, the possibility looms larger than ever that these receptors have a role in the natural history of herpes virus infections. It has been suggested by Westmoreland and Watkins and also by Costa and Rabson (*Lancet*, **i**, 77; 1975) that the development of F_c receptor sites may play a part in conferring a biological advantage on the virus and therefore in maintaining the latent nature of herpes infections, since the coating of infected cells with IgG molecules or immune complexes by the F_c region of the immunoglobulin molecules might protect the cells from the destructive effects of potentially cytotoxic antibodies and lymphocytes.

Lehner, Wilton and Shillitoe (*Lancet*, **ii**, 60; 1975) suggested an alternative way in which F_c receptors may be involved in the latent and recurrent nature of HSV infections, namely "double" binding of anti-HSV IgG antibodies to virus-infected cells to induced F_c receptors and HSV surface antigens, by way of their F_c and F_{ab} portions, respectively. The F_c portion of the anti-HSV antibodies would then be unavailable for either complement binding or for F_c receptors on effector cells capable of mediating antibody-dependent cell-mediated cytotoxicity. Lehner *et al.* also speculate that the spread of putative HSV-induced squamous cell carcinomas might also be facilitated by double-binding of F_c receptors and HSV antigens by IgG antibodies on the surface of dividing carcinoma cells. If there is indeed a relationship between HSV and squamous cell carcinoma, it will be of interest to see whether F_c receptors can somehow be exploited as a tumour cell marker in this particular case.

The *in vivo* significance of induced F_c receptors on herpes virus-infected cells awaits further experimentation. For the moment, the emphasis is on the various practical *in vitro* considerations of the phenomenon. A particularly illuminating example is the recent work of Rager-Zisman, Grose, and Bloom (*Nature*, **260**, 369; 1976) who found that HSV-infected target cells could be non-specifically lysed by F_c receptor-positive killer cells in the

absence of specific anti-HSV antibodies; the authors provided further evidence that this phenomenon might be mediated by the cross-linking of effector cells and target cells, both displaying F_c receptors, by aggregated immunoglobulins or soluble immune complexes present in the serum of the culture medium. This supports the suggestion first made by Lehner *et al.* (*op. cit.*) that complexes of HSV and IgG antibodies might bind to F_c receptors of both HSV-infected target cells and effector cells resulting in killing of the target cell.

Immune complex (or aggregated Ig)-dependent cell-mediated cytotoxicity may therefore represent one of the mechanisms by which F_c receptor-positive lymphocytes from normal donors can spontaneously lyse tumour target cells *in vitro*, a phenomenon observed by several investigators (for example, Pross and Jondal, *Clin. exp. Immun.*, **21**, 226; 1975; Bean *et al.*, *Cancer Res.*, **35**, 2902; 1975) and which has often been a serious technical problem in specific cell-mediated microcytotoxicity assays. If so, the use of F_c receptor-negative tumour target cells or culture media free of immune complexes and/or aggregated Ig should minimise or even prevent, in some cases, spontaneous lymphocyte-mediated cytotoxicity. □

Plant-microorganism interactions

from R. M. Cooper

A symposium on cell wall biochemistry related to specificity in host-plant pathogen interactions (organised by J. Raa and B. Solheim) was held at the most northerly university in the world in Tromsø, Norway on August 2-6, 1976. The proceedings of the meeting are to be published.

THE molecular basis of the specific parasitism or symbiotic associations of certain microorganisms with a plant species, or even variety, has fascinated but eluded scientists for many years. One such specific interaction is the so-called 'gene-for-gene' relationship between biotypes of plant pathogens and their hosts 'evolved' as a result of pathogens' overcoming new resistance genes introduced by man into crops. Similarly the symbiotic association between roots of legume species and strains of the soil bacterium *Rhizobium* (a major source of biologically-fixed nitrogen) can exhibit a marked degree of specificity. These two types of specificity comprised the

main theme of this excellent symposium.

Several examples of cell-cell recognition involve interaction of surface carbohydrates or glycoproteins of one cell and a protein of the other; such as mannoproteins in sexual mating in the yeast *Hansenula wingei* (C. Ballou, University of California), O-antigen in attachment of phage to bacteria, single terminal glycosyl residues as determinants of red blood cell types and glycoproteins in aggregation of sponge cells. Recently carbohydrates from cell wall surfaces of pathogenic fungi have been implicated in specificity by their ability to induce differentially antifungal compounds—phytoalexins—in resistant and susceptible host plant cells (Keen, *Science*, **187**, 74–75; 1975). P. Albersheim's group (including A. Ayers and B. Valent, University of Colorado) reported extensively on their findings with these 'elicitors'. Initially they isolated and partly characterised them from culture filtrates of *Colletotrichum lindemuthianum* (bean pathogen) and *Phytophthora megasperma* var. *sojae* (Pms) (soybean pathogen), and subsequently found them to be an integral part of the fungal cell wall as Pms elicitor could be released from walls using a technique designed to remove surface antigens from yeast. Purified Pms elicitor induced phytoalexin accumulation in soybean, at the same rate as during infection by Pms, when applied to plant tissue in extremely low amounts (10^{-13} mol). It is a small (less than 25 glycosyl residues) predominantly β -1,3-glucan, dependent for inducing activity on terminal sugar residues. Although elicitors may have a critical role in pathogenesis they do not seem to determine specificity, since : elicitors from different races of the two fungi appear identical; response of resistant and susceptible host varieties is similar; similar elicitors to Pms are found in two saphrophytic yeasts, which suggests that plants respond to microorganisms by recognising a few common elicitors. An exciting offshoot of this work could be the development of elicitors as non-toxic fungicides.

Agrobacterium tumefaciens induces tumours in a wide range of dicots and gymnosperms but not monocots. Pre-requisites for infection involve wounding of the host and attachment of virulent bacteria within 15 min of inoculation; avirulent strains are unable to attach. J. A. and B. B. Lippincott (Northwestern University, Illinois) found that the bacterial binding site resided within the polysaccharide moiety of the lipopolysaccharide (LPS) from the outer cell envelope; this fraction when isolated from virulent but not from avirulent strains inhibited tumour formation by competing with bacteria for host attachment sites,

Counting globin genes

from Pamela Hamlyn

THE molecular biology of abnormal human haemoglobins, and the study of haemoglobin variations within populations, not only provides explanations for clinically observed symptoms of diseases, but also acts as a model for the understanding of the biochemical genetics of other inherited traits. Simplicity is not one of the virtues of this particular model system. There are six main globins, α to ζ , which combine in different ways to form the various haemoglobins. The ϵ and ζ chains are present only in the first few weeks of foetal life.

Nucleic acid hybridisation techniques have shown that there are two genes coding for α -globin, one for β and one for δ . And now, to complete the count of the major globin genes, the number of γ -globin genes has been directly estimated (Old *et al.*, *Cell*, **8**, 13; 1976).

Two types of γ -chains are found in normal foetal haemoglobin, differing only at amino-acid position 136 which can be either alanine or glycine. The simplest interpretation of this would be that there are two structural genes for γ -globin. However, the proportions of the two types of γ -globin change after birth, and also γ -chain mutants do not occur in the ratio predicted if there are only two fully expressed genes. These observations have led to the suggestion that there are two major and two minor γ -globin genes (Huisman *et al.*, *Ann. N.Y. Acad. Sci.*, **165**, 320; 1969).

Because there are several steps between the transcription of the gene and the appearance of the protein, direct measurements of the number of genes are necessary; deductions made from the protein ratios are not sufficient. This was achieved by Old and his colleagues using a probe for γ -globin

genes. Messenger RNA from foetal red blood cells was reverse-transcribed to provide cDNA complementary to α , β and γ mRNA. Hybridisation of these cDNAs to adult blood mRNA, which contains only α and β mRNA, left the γ cDNA unhybridised and it was isolated from the hybrids. The purified γ cDNA was then hybridised to total human DNA in cDNA excess and the amount of cDNA needed to saturate the cell DNA was measured. The hybridisation of β -globin cDNA to total cell DNA was used as a comparison since it is known that there is one β -globin gene. The results confirmed that the number of copies of the β -gene was one, and showed that there were two genes for γ -globin. The different ratios between the two γ -globins which occur during development must therefore be due to differential gene transcription, or mRNA translation, rather than to gene dosage.

The method used in this study to determine gene number relies on the measurement of the rate and final extent of hybridisation with the probe in excess. This has a greater accuracy than the previously used method (Ottolenghi *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **72**, 2294; 1975) which relied on the measurement of the C_{ot} when cDNA was hybridised back to excess total DNA. This is because with the saturation method a doubling in the gene number results in a doubling of the final plateau value, whereas in the other method the estimate of gene number depends on a small difference in the C_{ot} normally measured on a logarithmic scale. Using the saturation method of determining gene number it will be possible to determine the frequency of globin gene duplication in human populations.

showing that LPS determined the specificity of attachment. The host component of attachment was in the cell wall as purified walls also inhibited tumour formation by competing for bacterial sites. The active fraction was polygalacturonide which was inhibitory at less than 1 ng ml^{-1} . In contrast monocot cell walls gave little or no inhibition of tumour induction, thus the inability of the bacterium to infect this group may result from lack of attachment sites. This is an interesting contrast to results of T. Graham and L. Sequeira (University of Wisconsin) on infection of tobacco by *Pseudomonas solanacearum*, in which avirulent strains attach to mesophyll cell walls and become rapidly enveloped by material, whereas virulent strains remain unattached and multiply freely. Binding may be determined by host

lectins (glycoproteins with diverse properties, for example haemagglutination) which agglutinated avirulent but not virulent cells because of the possession of extracellular polysaccharide by the latter.

Many other aspects of host-parasite interaction were considered, including plant cell wall structure, induction, repression and modification of pathogens' polysaccharidases, lignification, and degradation of fungal cell walls by host enzymes; however, these were discussed in terms of general rather than specific resistance. Some perhaps unexpected results were presented by H. Mussell (Boyce Thompson Institute, New York) implying interactions between pectic enzymes (PG) and host cell walls in specificity for *Verticillium* wilt of cotton; purified fungal PG induced symptoms only in susceptible

cotton cultivars. This may be partly due to the ability of 'resistant' walls to bind rapidly more PG than 'susceptible' walls, and the release of extra proteins, including peroxidase and IAA oxidase (both of which may be involved in symptom expression) from susceptible walls by PG.

Intriguing results were reported on host-symbiont interactions in which recognition and attachment of compatible rhizobia is the first stage before infection and nodulation of root hairs. Most evidence implied that recognition may depend on interactions between root lectins and bacterial surfaces. For example, W. D. Bauer (C. F. Kettering Research Laboratory, Ohio) found that soybean lectin only binds to living cells of symbiotic strains of *R. japonicum* and not to non-symbiotic strains, although there were some exceptions reported by J. Paxton (University of Illinois) and F. B. Dazzo (University of Wisconsin). Albersheim's results implied that the active bacterial surface component is LPS, as lectins from four legume species only interacted with the LPS of their corresponding symbionts. These lectins also had enzymic activity (against LPS) which may be a prerequisite for infection as intact LPS of *E. coli* elicits phytoalexin production; thus lectins may provide not only a physical linkage between surfaces but could trigger other critical events including a localised swelling of bacteria and decrease in respiration rate. Dazzo extended these studies using immunochemical techniques and found a surface antigen unique to infective strains of *R. trifolii* which cross reacted with antigens on roots of its symbiont clover; mutation to loss of infectivity resulted in loss of the antigens. A surface protein from clover roots, trifoliin, is specifically able to bind to the polysaccharide antigen and to agglutinate infective cells, and this probably explains specific adsorption by providing a bridge between the clover and rhizobial antigens. □

Antarctic ice and desiccation in the Mediterranean

from A. Hallam

ONE of the more spectacular results of the Deep Sea Drilling Project has been the confirmation that the deep Mediterranean basins are underlain by a thick layer of apparently shallow water late Miocene (or 'Messinian') evaporite deposits of the type previously known

from neighbouring land areas such as Italy. These evaporites are covered by Pliocene muds containing microfossils indicative of deep water conditions. For several years widely differing views have been put forward to account for such a peculiar deep sea occurrence. Hsu and his associates have argued that the northward convergence of Africa on Europe effectively cut off, about 6.5 million years ago, a series of deep topographic depressions in which, following desiccation, halite and calcium sulphate salts were precipitated in shallow salt pans many hundreds of metres below ocean level. Following the opening of the Straits of Gibraltar in the early Pliocene, Atlantic waters cascaded in to fill the basins and restore more or less normal oceanic conditions. Another school of thought rejects this interpretation, maintaining that the deep sea evaporites were originally deposited close to ocean level and occupy their present position as a consequence of substantial post-Miocene tectonic subsidence.

One means of deciding between these alternative hypotheses is to examine microfossils from deep sea core samples taken from the normal marine deposits directly below the evaporites. If these fossils are closely related to groups known to live at great depths in the present world ocean then it becomes difficult to argue that a shallow Miocene sea dried up and the present Balearic, Tyrrhenian, Ionian and Levantine basins were created by subsequent tectonic subsidence. In the proceedings of a micropalaeontologists' symposium held in Tunis (special issue of *Palaeogeography, Palaeoclimatology, Palaeoecology*, 20, 1/2; July 1976), Benson reports on ostracods and foraminifera obtained from sub-evaporite core material in the Balearic Basin, which appear to indicate unequivocally water depths in excess of 2,000 m. Similar microfossils have been found in contemporary sediments in the Guadalquivir Basin of southern Spain and suggest a continuous oceanic passage through that region from the Atlantic to the western Mediterranean in pre-Messinian times.

Most of the symposium was devoted to discussing the profound effect that the so-called Messinian 'salinity' or 'desiccation crisis' in the Mediterranean had on marine faunas. The pre-Messinian microfaunas, such as ostracods and planktonic and benthonic foraminifera, were very similar if not identical to Atlantic types. Mass extinction took place during the desiccation catastrophe but repopulation from the Atlantic source commenced in the early Pliocene once normal marine conditions were restored. The effects of the catastrophe on the evolution of individual species could well be

intriguing but has still not been fully evaluated.

Most scientists who have considered these extraordinary events in the geologically recent history of the Mediterranean have not sought for a world context, but there appears to be good support for Berggren's suggestion that the Messinian evaporitic phase corresponds with a global fall of sea level. Ecological analysis of foraminifera suggests a 40 m drop, which is in remarkably close agreement with the eustatic fall inferred from oxygen isotope data, due to a phase of growth of the Antarctic ice sheet at this time. It seems as though the structural and topographical situation of the Mediterranean region was such that a comparatively modest drop in sea level was sufficient to isolate it substantially from the world ocean, and well over a million years passed before a further eustatic rise allowed a normal connection to be renewed.



A hundred years ago

THE BRITISH ASSOCIATION

GLASGOW, Tuesday

THE Association finds a fitting home in Glasgow, which has few rivals either in earlier or later scientific reputation. The force of long-continued scientific traditions, added to the present encouragement given to science, and I must also say, to the nearness of the finest holiday localities, makes this one of the most brilliant of recent meetings. Not only is the total number of members and associates attending very high, over 2,700, but the true chiefs of science are present in great strength. It cannot be said that the Association itself is this year at all below its high aims. The majority of papers are really scientific, and do not emasculate the truth in the effort to popularise it. Discussions have been very interesting, judging from the perseverance with which they have been listened to. The reception given by the people of Glasgow is worthy of the city, although it is possible that in the details and refinements of arrangement, Bristol excelled. This was especially manifested in regard to some of the excursions. But it is evident that the very best efforts of the north have been put forth in every way, and the general result is undeniably successful. The charming situation of the University Buildings, in which all the sections but one hold their meetings, is a very great advantage. From *Nature*, 14, September 14, 425; 1876.

articles

Calculations for cancer radiotherapy with pion beams

J. E. Turner, R. N. Hamm & H. A. Wright*

Clinical trials with negative-pion beams for cancer radiotherapy will begin soon at four special facilities in the USA, Canada, and Switzerland. Negative pions are elementary particles which can be produced in high energy accelerators and which have properties that make them seem promising as a new way of treating tumours. The complex interactions that pions undergo in matter make it difficult to calculate dose patterns produced in a body irradiated by a pion beam and to solve that problem a unique combination of research in high energy physics and medicine is needed. Monte Carlo computer codes developed for radiation shielding and radiation protection studies have been adopted for use in dosimetry and treatment planning with negative pions. In this paper we briefly compare the properties of negative pions with those of other types of radiation and describe some calculations that we have made to help assess the potential of negative pions for cancer radiotherapy.

THE question of negative pions for treating cancer was first raised by Fowler and Perkins in 1961¹. Although negative pions have been available in some physics laboratories since the late 1940s², they have not been produced in sufficient intensity for radiotherapy and the large doses needed for treatment of tumours would have required impractically long irradiation times. Today, however, the first studies of pion radiation therapy are beginning in new facilities at the Los Alamos Scientific Laboratory, at Stanford University, at the Swiss Institute for Nuclear Research, and at the Tri-University Meson Facility in Vancouver³, as adjuncts to primary research programs in physics. Dose rates at these installations are many times greater than those available before.

Because of the expense and difficulty of producing pions in the laboratory, the number and type of experiments which can be performed to assess their potential for cancer therapy are limited. Furthermore, the complex interactions that pions undergo do not lend themselves to analytical calculations. But the availability of high speed computers with large core memories makes it possible to simulate, on the computer, the traversal of a beam of pions in tissue, and programs have been developed specifically to help in studies with negative pion beams in cancer radiotherapy.

Properties of the negative pion related to therapy

The existence of the pion was predicted on theoretical grounds by Yukawa in 1935 to account for the strong, short range nuclear forces that hold atomic nuclei together⁴. Pions, which have a rest mass 280 times that of the electron, can be charged or neutral and are rapidly destroyed through strong interactions with matter and through radioactive decay. They are produced in collisions of high energy particles. Although there were no machines in the 1930s capable of accelerating

particles to the required energies of several hundred million eV, it was recognised that sufficiently energetic cosmic rays bombard the Earth. The presence of the pion in cosmic radiation was confirmed in 1947 (ref. 5).

There are almost 100 known elementary particles and anti-particles, all of which might, in principle, be used for radiotherapy. Negative pions are now attracting attention because of their special physical properties. Like other charged particles, the depth of penetration of negative pions in a target can be varied by varying their energy, and they deliver a relatively high dose when they stop at the end of their track—in the so-called Bragg-peak region. Unlike most other charged particles, however, a stopped negative pion is attracted by a positive atomic nucleus and interacts strongly with it through the nuclear force. The nucleus absorbs the pion and literally explodes with 140 MeV of energy converted from the rest mass of the pion. Figure 1 shows an early cloud-chamber photograph of charged-particle tracks around a nitrogen nucleus that has captured a stopped negative pion. Nuclear capture at the end of its range is the special property that makes the negative pion of particular interest for therapy.

The fragments produced by the capture give additional dose to the matter surrounding the capture site (Fig. 1). The biological effectiveness of this dose is enhanced by the fact that a substantial fraction of it is deposited at high linear energy transfer, since high LET radiations are generally more effective in producing biological effects. Calculations indicate, for example, that some 15–20 MeV of energy is deposited in tissue within 0.2 cm of the capture site by particles with LET greater than that of a 2-MeV proton (170 MeV cm^{-1})⁶.

Furthermore, compared with low-LET, the effectiveness of high-LET radiation in killing cells is not reduced as much by the absence of oxygen. A rapidly growing tumour can presumably outgrow its blood supply and become hypoxic. In these conditions, it is well known that the dose of X rays or other low-LET radiations has to be two or three times higher to achieve the same degree of biological damage as in the aerobic state. Pions, in contrast, seem to offer a much more favourable

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Fig. 1 Cloud-chamber photograph of negative pion capture by nitrogen. The pion enters the picture from below and follows a curved path in the magnetic field through which it passes. The star at the end of the track is produced by two alpha particles and two protons. Four neutrons are also produced in the star but leave no visible tracks⁷.

"oxygen enhancement ratio". Hypoxic cells can be killed almost as effectively as oxygenated cells with high-LET radiation (for more detailed discussion see ref. 8 and references cited there).

Basis for clinical trials

In using radiation to treat a tumour, the objective is to deliver a large dose over a specified volume in the body and as little as possible elsewhere. In reality, however, the dose patterns obtainable are limited by the physics of the interaction of radiation with matter.

Fig. 2 shows a comparison of dose patterns in water along the axes of beams of ^{60}Co γ rays, 22-MeV X rays, protons and negative pions (based on Raju and Richman⁸). The levels of each kind of radiation are adjusted to give the same average dose over the interval between 7.5 and 12.5 cm. The energy spectra of the proton and pion beams were additionally adjusted to give a constant dose over this interval. The dose from ^{60}Co γ rays builds up in the first few millimetres in the absorber as the number of secondary electrons increases. The dose decreases beyond about 0.5 cm as the radiation is further attenuated and scattered. The curve for the more energetic X rays has a maximum near 3 cm and falls off less sharply with increasing depth. In contrast to the neutral radiations, energies of the charged protons and pions can be controlled so that they stop in the given region, where they deposit the highest dose. Whereas a stopped proton gives no further dose, a stopped negative pion produces additional dose through nuclear capture. The advantage gained by negative pions over protons is illustrated by the fact that the pion dose is considerably smaller between the surface of the target and the region between 7.5 and 12.5 cm.

The factors described so far constitute the theoretical basis for the research and investment in pions in radiotherapy. Medical rationale and plans for clinically treating specific types of neoplasms with negative-pion beams have also been developed⁹⁻¹¹.

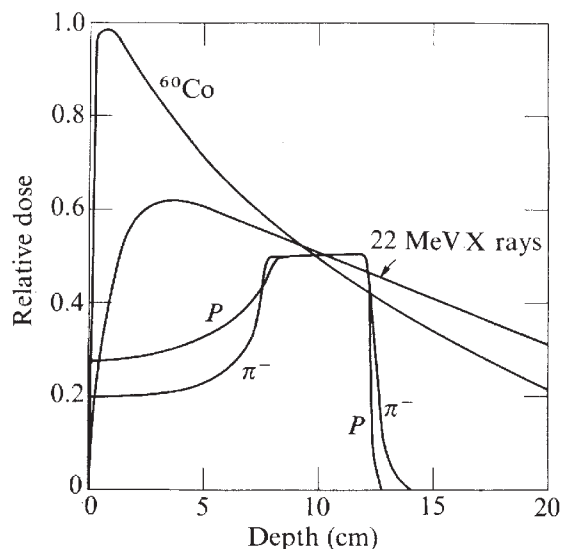
Computer codes for use in pion dosimetry

Several computer codes¹²⁻¹⁵ are available for making calculations for pion beams incident on a patient or tissue-equivalent phantom. Calculations of pion-dose distributions pose several problems not encountered for other charged particles or conventional X or gamma rays. Because of the violent reactions that pions undergo, dose in the phantom does not readily lend itself to a description by means of simple analytical formula. Knowledge of the dose pattern from one beam-target configuration is of limited usefulness in predicting dose under different conditions. The variety of secondary particles produced—each with its own special properties—also complicates any analytical representation.

We have used a Monte Carlo technique to simulate on a computer the statistical distribution of events which occur to the individual particles in the phantom. By treating several thousand incident particles and the secondary particles they produce, one can thus obtain a detailed physical picture of what occurs in actual experimental conditions. The reliability of this picture depends directly on the reliability of the experimental data used in the calculations.

PION-1 was designed to treat all processes which pions undergo with sufficient accuracy to simulate real pion transport for radiotherapy applications. (It was developed in the Health Physics Division at ORNL and is at present restricted to pions, muons and electrons, with energies up to 125 MeV in materials consisting of H, C, N, and O. Other computer codes, developed in the Neutron Physics Division, are designed to transport pions, muons, protons and neutrons with energies up to hundreds of GeV. These codes use an intra-nucleus cascade model to calculate nuclear reaction products. PION-1, which makes use of some data calculated by the Neutron Physics codes, runs considerably faster than the more general, multi-purpose codes.) Experimental values of the cross sections have been used to determine pion-nucleon interactions. These values can be revised or supplemented should data become available. All relevant pion nuclear reactions are programmed into the code: pion absorption, one- and two-nucleon knockout, and charge exchange. When an incident pion enters the phantom, the Monte Carlo technique is used to determine its fate, on the basis of these cross sections. As the pion penetrates the phantom, it undergoes multiple Coulomb scattering and also slows down in accordance with the stopping-power formula for charged particles. Range straggling is also included. When a nuclear reaction occurs, the code selects the type of reaction and the

Fig. 2 Examples of dose patterns in water along the axis of beams of ^{60}Co γ rays (1.33 and 1.17 MeV), 22-MeV X rays, protons (P), and negative pions (π^-).



energies and initial directions of travel of any secondary particles produced. The secondary particles are also followed individually in the calculations. The use of numerical data, rather than nuclear models, allows the program to process particles rapidly.

For analysis, the phantom is divided into a number of subvolumes. A record is kept in the program of each particle that traverses or stops or starts in every subvolume. The energy deposited, the LET of the radiation, and other physical quantities of interest are tabulated—the Monte Carlo method gives complete physical detail. Parameters for use in cell survival models can also be tabulated. Such data would be prohibitively expensive and difficult to obtain experimentally.

Within the context of negative-pion therapy, PION-1 is versatile. No special assumptions about the beam and phantom geometries are necessary. Pions can have any pattern of incidence on the phantom and any energy distribution up to 125

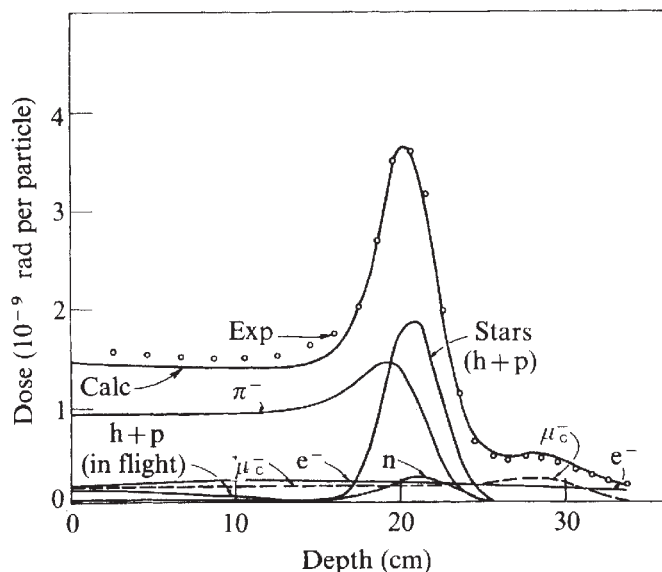


Fig. 3 Total dose, calculated (smooth curve) and experimental (circles), for CERN negative-pion beam. Radius of the detector was 3 cm. Other curves show various calculated contributions to the total dose. Labels on the curves indicate doses from the following sources: pion ionisation (π^-), heavy particles and protons generated from interactions of pions in flight ($h+p$), muon contamination (μ_c^-), electron contamination (e^-), heavy particles and protons generated from interactions of stopped pions (stars), and neutrons (n). The beam was assumed to be composed of 63% π^- , 23% e^- , and 14% μ^- with mean momentum 176 MeV/c (energy 85 MeV) and momentum spread 2.0%.

MeV. (The program can be extended to higher energies, if desired.) Muons and electrons can be present. The phantom can be composed of water, tissue, or other tissue-like material and can have regions of bone, lungs, air cavities, and so on.

The code runs rapidly. In a typical calculation, 10^4 incident pions and all the secondary particles they produce are processed completely. The statistical data are then excellent for plotting depth-dose and isodose contours and for compiling LET distributions and parameters for cell survival. The complete run takes about 10 min on the IBM 360/91. The processing of neutrons requires by far the most time. Without neutrons, the running time for 10^4 pions and the other secondary particles is less than three minutes. In many applications we include neutrons in only a fraction of cases.

Comparison of measurements and calculations with PION-1

Ideally, any calculation technique should be substantiated through detailed experimental checks of its component parts. Because of the complexity of pion interactions, detailed veri-

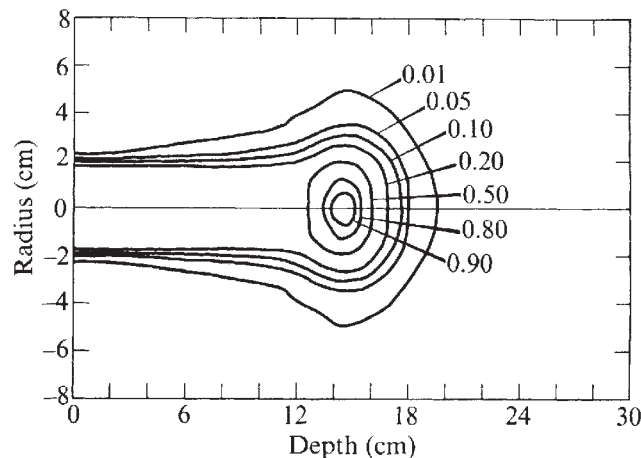
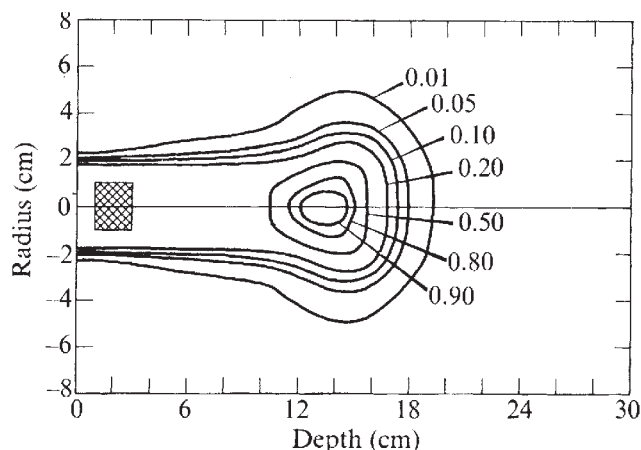


Fig. 4 Isodose contours for pion beam incident on soft-tissue target.

fications are not feasible. Many of the experiments would be costly and time consuming, while others would be prohibitively difficult.

Some direct comparisons have been made between measured doses and doses calculated with PION-1. Figure 3 shows one such comparison made for an experimental negative-pion beam from the 600-MeV synchrocyclotron at the European Centre for Nuclear Research (CERN) in Geneva, Switzerland. This work was carried out as a collaborative effort between the Health Physics Division at the Oak Ridge National Laboratory and the Health Physics Group at CERN¹². The top curve, marked 'Calc', represents the dose calculated as a function of depth in a water target used in the experiment. The open circles, designated 'Exp', represent the values of the dose measured experimentally. The computations were performed for a beam mixture of 63% negative pions, 23% electrons, and 14% negative muons. A Gaussian momentum distribution was assumed with a mean value of 176 MeV/c (energy of 85 MeV) and a spread of 2% (that is, half of the particles have momentum within $\pm 2\%$ of the mean). The beam, which was non-uniform, was approximately elliptical in shape with major and minor axes of length 7 and 6 cm. The circular cylindrical detector of radius 3 cm, placed at different depths along the beam axis in the experiment, was simulated in the computations. The calculated individual contributions, which add up to the total dose 'Calc', are also shown in Fig. 3. The curve marked ' π^- ' gives the dose that results from the direct ionisation of atoms by the pions in slowing down. The curve ' $h+p$ ' shows the dose from heavy recoil particles (atomic mass number > 1) and recoil protons produced by pions in flight. (About 15% of the incident pions

Fig. 5 Isodose contours for pion beam incident on soft-tissue target with bone cylinder between 1 cm and 3 cm in the cross-hatched region.



react with nuclei in the target before they reach the end of their range.) The local dose in the stopping region from the charged particles produced by pion capture is shown by the curve labelled 'Stars'. Neutrons, which occur primarily as a result of pion capture, give the dose shown by the curve 'n'. The doses from the muons and electrons in the experimental beam are shown by ' μ_e^- ' and ' e^- '.

This comparison indicates that the dose from a beam of negative pions can be calculated from the known physics of pion interactions with some degree of confidence. Uncertainty in the calculated dose seems to be comparable to the reproducibility of the experimental measurements. Although direct experimental comparisons with most of the individual contributions shown in Fig. 3 have not been made, the curves are consistent with known physical data. The calculated number and energy spectra¹⁶ of the particles produced by stopped-pion capture by oxygen have been compared with measurements¹⁷ and seem to be in reasonably good agreement.

Dose patterns with negative pion beams

Of more direct interest for therapy are the dose patterns expected in tissue irradiated by different pion beams. Figure 4 shows calculated isodose contours in a tissue target of unit density from a parallel, uniform circular beam (radius 2 cm) of negative pions, having a Gaussian momentum distribution with a 2% spread and a mean energy of 67 MeV. The pions have a mean range of 15.0 cm. The beam is incident from the left, the axis being the line marked 0. The contours have cylindrical symmetry about the beam axis and apply to any plane containing that axis. The levels are shown as fractions, varying from 90% to 1% of the peak dose, 1.79×10^{-8} rad per incident pion, which occurs at a depth of 14.5 cm. The dose is not confined completely to the region directly intercepted by the beam. The beam spreads as it traverses the target because of multiple Coulomb scattering and nuclear reactions, which scatter particles outside the immediate beam area. The neutrons produced can deposit dose many centimetres away from the beam axis. Repeating the calculations but ignoring the neutrons gave virtually the same contours for the levels of 5% of the peak dose and greater. The peak dose itself was reduced, however, to 1.71×10^{-8} rad per pion. The lateral spread of the 1% contour from the beam axis at a depth of 15 cm changed from 4.9 to

4.4 cm when neutrons were neglected.

The presence of air cavities, bone and other regions of different densities in the body can greatly affect dose patterns. Figure 5 shows an example of dose contours when bone (density, 2 g cm^{-3}) is present. The bone is in the form of a right circular cylinder of radius 1 cm, centred on the beam axis between the depths 1 cm and 3 cm, as indicated by the cross hatching in Fig. 5. The same pion beam was used in the calculations of Figs 4 and 5. The peak dose with the bone present is decreased to 1.46×10^{-8} rad per incident pion and occurs at a depth of 12.6 cm as compared with 14.5 cm before. The bone increases the amount of multiple Coulomb scattering and some additional nuclear reactions, causing the dose pattern to be more diffuse in Fig. 5. A systematic study of the effects of tissue inhomogeneities has been completed recently^{18,19}.

Another complication, which must be assessed in using pions for radiotherapy, results from the neutrons produced from pion capture. The neutrons will travel away from the capture site in a tumour and irradiate healthy tissues elsewhere in the body. The neutron dose delivered to a sensitive organ in the vicinity of a tumour may limit the therapeutic dose that can be given to the tumour. We are now calculating the dose contributions from neutrons for a variety of pionbeam configurations and tumour sizes.

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The origin of deuterium

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General nuclear constraints are used to show that deuterium is most likely of pregalactic origin. Big-bang nucleosynthesis is the most plausible source for significant amounts of this isotope, but other, more speculative, sources are not ruled out.

THE amount of deuterium which is formed during the initial phase of a standard hot big-bang universe is a sensitive function of the mean baryon density ($X_D \propto \rho^{-5}$ over the range of interest; X_D is the deuterium mass fraction and ρ is the mean baryon density; (see ref. 1). If big-bang nucleosynthesis were the only significant source of deuterium, the amount of deuterium

produced by this event would have to be at least as great as that which is currently observed, $X_D \sim 2 \times 10^{-5}$ (refs 2 and 3). The mean mass density of the present Universe would then be $< 6 \times 10^{-31} \text{ g cm}^{-3}$ and hence less than the Einstein-de Sitter critical density $\rho_c = 3H^2/8\pi G = 4.5 \times 10^{-30}(H/50)^2 \text{ g cm}^{-3}$ (H is the Hubble expansion parameter in $\text{km s}^{-1} \text{ Mpc}^{-1}$). In the framework of Friedmann cosmologies with cosmological constant $\Lambda = 0$ this would mean that the Universe is open and forever expanding^{4,5}. Conversely, if the mean density of matter in the Universe is appreciably $> 6 \times 10^{-31} \text{ g cm}^{-3}$, the current deuterium abundance could not have been produced by nucleosynthesis during the early phase of a standard universe. These conclusions are insensitive to quite substantial deviations

from the standard cosmological models⁶, presuming also that the bulk of the observed ⁴He was produced in the big bang.

These statements provide good reason for investigating other possible sources of deuterium. If an exhaustive study yields no physically self-consistent or at least plausible models for deuterium production, then this failure lends support to the notion that deuterium is of cosmological origin. On the other hand, if an eminently plausible source of deuterium production or enhancement could be found, then the current deuterium abundance would not necessarily be related to the physical conditions in the early Universe and hence could not be considered to be an important cosmological discriminator.

We examine here various non-cosmological mechanisms which might be capable of producing significant amounts of deuterium. First, the general nuclear constraints which must be satisfied by all viable models for deuterium production are summarised. Specific models for deuterium sources are then discussed and critically analysed. The net result of this investigation is that very severe restrictions can be placed on mechanisms for producing deuterium. In fact, it seems unlikely that objects or events which are currently known or inferred to have existed in our Galaxy or in well-observed extragalactic objects could be capable of producing the observed deuterium abundance.

Overview

Deuterium formation can occur either through synthesis reactions in which free nucleons fuse together or by means of spallation or photodisintegration reactions in which energetic particles disrupt more massive nuclei, thereby ejecting deuterons. All models of deuterium production invoke one or both of these processes.

Synthesis models run into difficulty because the conditions which are needed for the making of deuterium are normally the same as those for its rapid destruction. Deuterium can be synthesised by ¹H(n,γ)D reactions if free neutrons and protons are able to interact. Free neutrons are produced at high temperatures, when heavier nuclei thermally dissociate, but any deuterium which is so formed is liable to be consumed in thermonuclear reactions. Deuterium could survive a hot explosive event only if the hot matter is dispersed and/or cooled rapidly compared with the time which is required for deuterium destruction. It is shown below that an object of even moderate mass ($\gtrsim 10^{-7} M_{\odot}$) cannot expand rapidly enough for significant amounts of deuterium to survive. Free nucleons may also be produced by the passage of a strong shock wave⁸⁻¹⁰. In this type of process the gas cools quickly enough for the deuterium to escape destruction, but models which invoke shock waves for deuterium production are faced with a number of other difficulties¹¹⁻¹⁵ which we also discuss below.

The possibility that a disrupting neutron star could provide a cool flux of particles has been examined (refs 16, 17 and J. M. Latimer and D. N. Schramm, unpublished), but it was found that this type of event cannot simultaneously provide both free neutrons and free protons, and hence cannot produce deuterium. Cameron and Truran (unpublished) have suggested that if hot neutron-rich material could be ejected by MHD processes from a collapsing stellar core^{30,31}, deuterium production could result. The constraints on this type of model are discussed below.

Spallation

Models which involve the production of deuterium by spallation reactions are faced with the problem that under most conditions the concomitant production of other light nuclei or γ rays is much too rapid. This is an especially strong constraint because deuterium is destroyed by thermonuclear reactions more easily than any other stable nucleus; any subsequent processing of the source material can only decrease the abundance of deuterium relative to other nuclei.

The interaction of energetic particles with normal population-I composition forms deuterium mainly by $p + \alpha$ reactions.

Other light nuclei, such as Li, Be and B, are formed by the interaction of protons or α particles with C, N, or O nuclei and by $\alpha + \alpha$ reactions, and γ rays are formed by high energy $p + p$ reactions producing π^0 which subsequently decay to two γ rays, each with an energy ~ 70 MeV. By considering the nuclear reactions which can occur in a hydrogen-helium gas mixture only, we can obtain very strong constraints on deuterium source models; these constraints are applicable to deuterium production in the present epoch and in pregalactic but post-helium synthesis epochs. (Deuterium production from spallation is impossible before helium synthesis.)

The rate at which a particle of type i is produced by the interaction of particles j and k is

$$\frac{dY_i}{dt} \propto Y_j Y_k \sigma_{i,jk}(E)$$

Here Y represents the number fraction of a particle (Y_i is the number of particles of type i per nucleon; $Y_i = X_i/A_i$ where A_i is the atomic weight), $\sigma_{i,jk}$ is the production cross section and E is the energy per nucleon of the incident particle.

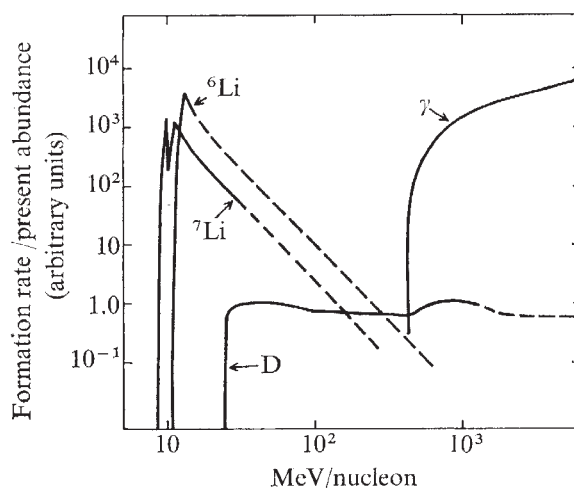
The relative production rate, which is defined by

$$R_i \equiv \frac{1}{Y_{i,\text{present}}} \frac{dY_i}{dt} \quad (1)$$

gives the rate at which the abundance of a particle approaches the present value. When two nuclei have the same value of R , their abundance ratio at the source is the same as their present cosmic abundance ratio.

The relative production rates for deuterium, ⁶Li, ⁷Li and γ rays are shown in Fig. 1 as a function of the energy per nucleon of the incident particle. The system is assumed to contain initially only hydrogen and helium with Y_{α}/Y_p taken to be 0.07 in the energetic particle flux and in the ambient medium. The present densities of the other particles relative to deuterium are $Y_{\text{Li}}/Y_D \approx 10^{-5}$, $Y_{\text{Li}}/Y_D \approx 10^{-4}$ and $Y_{\gamma}/Y_D \approx 10^{-3} \rho_c/\rho$ (refs 18, 19). The last ratio is determined by using the number of photons in the diffuse background with energies > 70 MeV and assuming that the galactic deuterium abundance $Y_D = 10^{-5}$ is universal. It is important to note that in the present epoch there is far more deuterium than there is ⁶Li, ⁷Li or γ rays. Any class of events which produces even a relatively small amount of these particles cannot be the dominant source of the galactic deuterium.

Fig. 1 The rates at which abundances approach their present values as a function of the energy per nucleon of the incident particle. See equation (1) and text. Data are from refs 19, 28, 29 and C. King, private communication. Dashed lines are extrapolations from known data.



This constraint is clearly shown in Fig. 1. Particle fluxes with energies $\lesssim 300$ MeV per nucleus generate very large $Y_{\text{Li}}/Y_{\text{D}}$ ratios, and particles with energies > 500 MeV per nucleon produce high Y_{γ}/Y_{D} ratios. Models for a spallation origin of deuterium must rely on a flux of low energy particles and some mechanism for preferentially destroying the lithium or on a flux of high energy particles and some means of absorbing the γ rays photons. Particles with a narrow spread of energy ~ 400 MeV per nucleon could possibly produce reasonable $Y_{\text{Li}}/Y_{\text{D}}$ and Y_{γ}/Y_{D} ratios, but models which require nearly monoenergetic particles are rightly considered *ad hoc* and are hard to justify on physical grounds.

It has been pointed out that the γ rays which form from the decay of π^0 can cause the photodisintegration of ^4He and thereby produce deuterium²⁰. However, the γ rays preferentially produce electron-positron pairs^{21,22} so that only a few percent of these photons can ultimately produce deuterium. Since $Y_{\gamma}/Y_{\text{D}} \sim 10^{-3}$ at present, any model which invokes photodisintegration to form deuterium must also contain some mechanism for γ -ray destruction. Furthermore, if the π^0 are formed by high energy nuclear collisions, most of the deuterium is actually formed directly by $p+\alpha$ collisions, so that the photodisintegration reaction is of only minor importance.

Finally, it should be re-emphasised that, in the above discussion, the reactions on nuclei heavier than ^4He have been omitted. The inclusion of even a small amount of C, N or O nuclei would make it much more difficult to formulate an acceptable model for deuterium formation by spallation. A heavy element mass fraction $\sim Z = 10^{-4}$ leads to overabundances of Li, Be and B relative to deuterium. This effectively eliminates such events as present-day solar flares³².

Pregalactic cosmic rays

The high energy particles in the Galaxy could not have produced the galactic abundance of deuterium. The total number of these particles which have existed throughout the age of the Galaxy is far below what is required to produce significant amounts of deuterium, and one is still faced with the difficulty that lithium and other light nuclei would be overproduced. Rather than deal with the restrictive problem of whether deuterium could be formed in any particular type of event, consider the more general question: can one formulate any physically self-consistent model for a cosmic-ray origin of deuterium? The simplest possible model involves 'pregalactic cosmic rays' (only protons and α particles) of arbitrary energy interacting with an ambient medium of hydrogen and helium. A study of this sort has recently been undertaken by Epstein (unpublished) where particle spectra of the form

$$F(E) \propto (E + E_B)^{-2.5} \quad (2)$$

were used, with E_B an adjustable parameter. This spectral form has the advantage of being a simple one-parameter function which reduces to approximately the galactic cosmic-ray spectrum for suitable values of E_B ($\lesssim 1$ GeV per nucleon). Essentially similar results are obtained by using power law spectra of various indices and with high and/or low energy cutoffs.

When these cosmic rays interact with the ambient medium, deuterons, lithium nuclei, γ rays and other particles are formed. The initial $Y_{\text{D}}/Y_{\text{eLi}}$ production ratio is shown in Fig. 2 as a function of E_B (this ratio is used because it provides a stronger constraint than $Y_{\text{D}}/Y_{\text{Li}}$). Only for $E_B \gtrsim 20$ GeV per nucleon is the initial value of $Y_{\text{D}}/Y_{\text{eLi}}$ comparable to the observed ratio. As the system evolves, the proton and α particle spectra are degraded by ionisation losses and nuclear collisions, the deuterons and lithium nuclei which were produced at high energies are slowed down or destroyed, and some of the γ rays are absorbed by the ambient medium. When all relevant production, destruction and energy loss processes are included the $Y_{\text{D}}/Y_{\text{eLi}}$ ratio evolves, finally reaching the values indicated

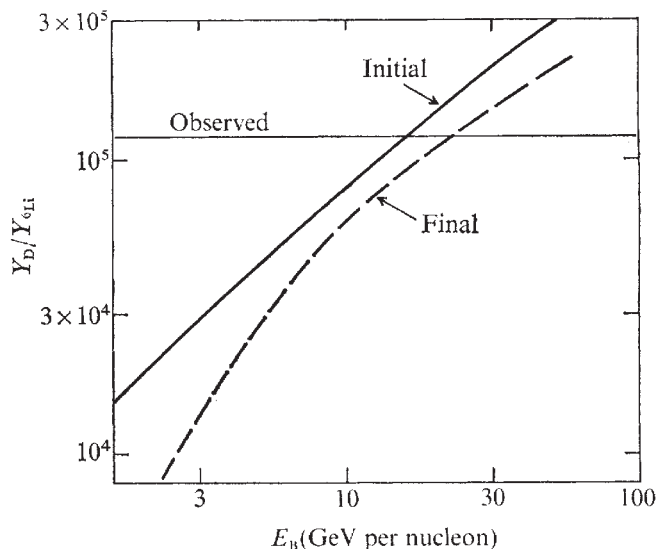


Fig. 2 Ratio of the abundances of deuterium and ^6Li which are produced by spallation reactions. The solid line is for spectra defined by equation (2). The dashed line is for spectra which are initially given by equation (2) and subsequently evolve. The observed abundance ratio is indicated.

by the dashed line in Fig. 2. Only for values of $E_B \gtrsim 30$ GeV per nucleon is the final $Y_{\text{D}}/Y_{\text{eLi}}$ ratio acceptable. For these very hard cosmic ray spectra γ rays are, however, overproduced by a factor of $> 10^4$ relative to the diffuse background. Only if the source of γ rays were shielded by $\sim 500 \text{ g cm}^{-2}$ of matter would the observable flux of γ -ray photons be consistent with existing measurements. The energy which is required to give a deuterium abundance of $X_{\text{D}} = 2 \times 10^{-5}$ is $1.7 \times 10^{18} \text{ erg g}^{-1}$, or $5 \times 10^{62} \text{ erg}$ for a $10^{11} M_{\odot}$ galaxy.

A spallation origin of deuterium cannot be totally dismissed on the basis of the above discussion, but can the necessary conditions for this mode of deuterium formation be realised in any reasonable astronomical event? The intense flux of cosmic rays, the low heavy element abundance and the large column density are certainly inconsistent with what is known about normal galaxies. Quasars, on the other hand, emit roughly the correct amount of energy, much of which is presumably derived from non-thermal particles, and they could provide sufficient shielding (a $10^8 M_{\odot}$ object with a 6-pc diameter has a column density $> 10^3 \text{ g cm}^{-2}$). Observed quasars contain elements heavier than ^4He , but an earlier generation of 'extreme population II' quasars could be a possible site for deuterium production. The absorption of γ rays could also be achieved by the general ambient medium of the Universe if deuterium formation occurred at a sufficiently early epoch. This would require that deuterium production occur at redshifts $> z = 300$, shortly after recombination of the ionised matter in the Universe²³. One can conjecture that an intense flux of high energy particles could be generated by an early generation of collapsing objects²⁴ or by energetic turbulence which results from the readjustment of the cosmic gas after its decoupling from the radiation field. Without other supportive evidence for such mechanisms, however, their invocation seems, again, quite *ad hoc*.

Shock waves

It has been suggested by Colgate^{8,9} and by Hoyle and Fowler¹⁰ that strong shock waves propagating through a low density gas could produce deuterium. The basic idea is that as a shock wave propagates through a low density medium, such as an extensive stellar envelope, it can exhibit a brief non-equilibrium phase in which high energy ion reactions ($\gtrsim 10$ MeV per nucleon) can occur; this 'ion precursor' phase arises partly from the initial collisions between the outwardly streaming ions and the

stationary low density matter, and partly from the relatively long time (compared to a nuclear reaction time) that is required for the shocked gas to relax to thermal equilibrium. As equilibrium is attained through electron heating and photon emission processes, most of the internal energy of the shocked material is transferred to the radiation field and the ions are quickly cooled. During the ion precursor, spallation reactions of protons on helium produce deuterium and free nucleons while spallation reactions on the CNO nuclei and α - α reactions produce Li, Be, and B. In the cool gas behind the precursor the free nucleons recombine to augment the deuterium abundance. Though this mechanism is an ingenious blend of the two basic deuterium production processes it runs into difficulties on several accounts.

First, the very existence of strong ion precursors is in doubt. Weaver¹⁴ has been able to obtain solutions only for the shock wave structures which do not exhibit high temperature precursors. Furthermore, no one has been able to show that shock waves with ion precursors are actually physically self-consistent.

The second problem is that shock-wave nucleosynthesis tends to overproduce some of the light elements relative to deuterium^{11,12}. Shock waves of energies up to 50 MeV per nucleon produce deuterium and other nuclei by direct spallation so that ⁷Li is always overproduced. Stronger shock waves, however, spall nearly all the nuclei to free nucleons, thereby destroying the ⁷Li overabundance and allowing for the subsequent synthesis of deuterium by ¹H(p,n)D. The unresolved question is whether an appreciable amount of matter can be processed by very strong shock waves without an even larger amount of matter being exposed to weaker shock waves which would preferentially produce ⁷Li. Colgate²⁵ contends that at low shock velocities the diffusion of radiation damps the development of a precursor (and thus prevents ⁷Li from forming), and that at high shock velocities the radiation does not keep pace with the shock wave so that the precursor develops and deuterium forms. As mentioned before, the detailed calculations of Weaver do not support this picture.

If the arguments in favour of deuterium production in shock waves were accepted there would still remain the question of what the origin of these shock waves could be. Normal supernovae appear to be highly unlikely sites. Using the most favourable assumptions (low CNO abundances, efficient acceleration of the shock waves) Epstein *et al.*¹² found that energies $> 10^{53}$ erg would have to be released in a typical $5M_{\odot}$ type II supernova; this is far greater than any observational inferences¹⁵. Pregalactic supermassive objects could have, perhaps, undergone extremely violent explosions, and few constraints can be put on their energetics. Nevertheless, there is no reason to expect that they could be any better suited for producing deuterium than current supernovae.

Hot explosions

Deuterium can be formed if matter is brought to high temperatures where heavy nuclei decompose to free nucleons ($T > 10^9$ – 10^{10} K, depending on density) and then rapidly expanded and cooled. The deuterium is formed by ¹H(n, γ)D reactions, and it is also destroyed by thermonuclear processes, the most important destruction reactions being (D(d,n)³He and D(d,p)³H as long as $Y_D \gtrsim 10^{-5}$. What fraction of the deuterium which is formed in an explosive event actually escapes destruction? If the densities of neutrons and protons are nearly equal there are two possible sequences by which deuterium can be formed and also survive (the case in which there is a large excess of neutrons is considered later):

(1) The expansion may be rapid enough so that some neutrons and protons do not recombine until the gas cools to the 'freeze-out' temperature ($T \lesssim 3 \times 10^7$ K), when the charged-particle reactions are slower than the expansion. The deuterium which is subsequently formed is ejected without much thermonuclear destruction.

(2) Deuterium forms while the gas is still hot but the expansion

is rapid enough so that deuterium is ejected without being completely destroyed.

Neutrons and protons are consumed at a rate

$$\frac{dY_n}{dt} = \frac{dY_p}{dt} = -4 \times 10^4 \rho Y_n Y_p \text{ s}^{-1} \quad (3)$$

where ρ is in g cm^{-3} . Define $\rho_0 \tau_{\text{exp}} \equiv \int \rho dt$ where the integration is from the time at which the nucleons start being consumed (when $T \gtrsim 3 \times 10^9$ K) until freeze out. The upper limit on the number of free neutron-proton pairs at freeze out and hence on the abundance of deuterium which forms after freeze out is

$$Y_D \lesssim (4 \times 10^4 \rho_0 \tau_{\text{exp}})^{-1} \quad (4)$$

Deuterium which forms at high temperatures is subject to destruction by thermonuclear reactions. The rate at which deuterium is burnt by D+D reactions at $T \sim 10^9$ K is²⁶

$$\frac{dY_D}{dt} \simeq -10^7 \rho Y_D^2 \quad (5)$$

so that the amount of deuterium which forms at high temperatures and survives until freeze out is limited by $Y_D \lesssim (10^7 \rho_0 \tau_{\text{exp}})^{-1}$. Since this is far less than the maximum amount of deuterium production after freeze out, equation (4) represents a strong upper limit to the net deuterium production in a hot explosion.

Since only a small fraction of the matter in the Universe could have been raised to high temperatures and then rapidly expanded (except, of course, in the big bang), the deuterium fraction in the ejecta has to be greater than the interstellar value of $\sim 10^{-5}$. Taking the value to be $Y_D > 10^{-3}$ and using equation (4) gives

$$\rho_0 \tau_{\text{exp}} < 2 \times 10^{-2} \text{ g s cm}^{-3} \quad (6)$$

Can such rapid expansion rates be attained in realistic explosive events? To obtain a lower limit to τ_{exp} , consider an homogeneous sphere of mass M and density ρ expanding so that its surface moves with some speed less than the velocity of light. Taking $\tau_{\text{exp}} = \rho/(d\rho/dt)$ gives

$$\rho \tau_{\text{exp}} \gtrsim 0.9 \rho^{2/3} (M/M_{\odot})^{1/3} \text{ g s cm}^{-3} \quad (7)$$

The density of matter at 10^9 K has to be $\gtrsim 8 \text{ g cm}^{-3}$ for the energy density of the radiation $> \rho c^2$. (Only in the big bang can this condition be reasonably violated.) Expansion rates which are consistent with equation (6) thus require the mass of the exploding region to be $\lesssim 10^{-7} M_{\odot}$. Since this is much too small to correspond to any reasonable astronomical event, we conclude that significant amounts of deuterium cannot survive a hot explosion.

Disrupted neutron stars

Can neutron stars provide the free neutrons which are needed for deuterium synthesis? Lattimer and Schramm (ref. 16, and unpublished) investigated a mechanism whereby a neutron star falling towards a black hole (for example, a binary system decaying by gravitational radiation) is tidally disrupted and ejects some neutron-rich matter from the system (the 'tube-of-toothpaste' effect), and others (refs 30, 31 and A. G. W. Cameron and J. Truran, unpublished) have shown how neutron-rich matter can be ejected by MHD instabilities from the collapsing core (a 'hot neutron star') of a highly evolved star. For deuterium to be produced in this type of process the neutron-rich matter has to interact with protons before (1) all the free neutrons are consumed in forming heavy nuclei or (2) before the neutrons decay to protons. Following Lattimer *et al.*¹⁷ it seems that, as the neutron-star matter expands, the few protons which are initially present ($Y_p \simeq 0.03$ – 0.04) clump

together with the neutrons to form clusters. The excess neutrons 'boil off' until the nuclear clusters reach the neutron drip line. As the density decreases further, β decays begin to occur in the nuclei; the increase in proton number permits the recapture of neutrons. Nuclei up to mass $A \sim 300$ are formed and then fission produces more seed nuclei. Neutrons are consumed as rapidly as permitted by the β decays and fissions of the heavy neutron-rich nuclei. The timescale for this to occur is ~ 0.1 s. (Similar processes occur for the hot neutron star except the initial Y_p are 2 or 3 times larger and the neutron depletion time scales could be considerably longer.) Expansion rates more rapid than this would allow some free neutrons to escape.

Even if some free neutrons do escape, however, they decay in $\sim 10^3$ s and do not produce any deuterium unless they interact with hydrogen-rich matter with a density of at least 10^{-8} g cm $^{-3}$. Densities of this magnitude are associated with stars or stellar envelopes. Models of deuterium formation which require a large number of neutron stars to interact with black holes and to spew neutron-rich matter into nearby stars are probably beyond the range of reasonable speculation. Hot neutron stars, however, are still surrounded by the pre-supernova stellar envelopes. For the hot neutron-rich material to be able to interact with the overlying hydrogen mantle several conditions must be satisfied: (1) the β -decay rates for very neutron-rich nuclei must be ~ 1 s; (2) the neutrons cannot interact with the heavy elements which surround the core, and (3) the neutron-rich matter has to be ejected rapidly enough so that it can reach the hydrogen mantle (a distance of 10^{10} – 10^{12} cm from the core) in $< 10^3$ s. Any or all of these conditions are likely to be violated, but for now this remains a viable model.

Conclusion

Big-bang nucleosynthesis provides an elegant theory for the synthesis of deuterium and helium. It requires only the simplest cosmological assumptions and is consistent with all well established astronomical data. Contrasted to this, post-big-bang deuterium production requires extremely violent and exotic events, the existence of which is certainly doubtful. From the perspective of our current understanding of the chemical evolution of the Universe, big-bang nucleosynthesis is by far the most reasonable site for the origin of deuterium. What are the prospects that this conclusion may be altered or made more firm? Two approaches may be fruitful. First, one can search for direct evidence of post-big-bang deuterium formation such as inhomogeneities in the abundance of deuterium or enhancements in the abundances of lithium, beryllium, boron and γ rays, which may be associated with deuterium production. Variations of this sort either in our Galaxy (ref. 27, and A. A. Penzias, P. G. Wannier, R. W. Wilson and R. A. Linke, unpublished) or in extragalactic objects such as quasars (T. Adams, unpublished) would be of great importance. The results to date

indicate a possible decrease in the deuterium abundance near the galactic centre (A. A. Penzias, P. G. Wannier, R. W. Wilson and R. A. Linke, unpublished); this is consistent with any pregalactic origin of deuterium followed by destruction of deuterium by stellar processing. The second approach, which is being vigorously pursued by a number of researchers (see ref. 5 and references therein), is to determine the local mean mass density of the Universe. Considerations of the dynamics of galaxies and groups of galaxies has led to the tentative result that the mean density is at least 5×10^{-31} g cm $^{-3}$. If further studies could raise this limit by even a firm factor of two or three (still leaving ρ below the critical density ρ_c), then the standard cosmological picture for the origin of deuterium would have to be seriously questioned and exotic pregalactic or hot neutron star models might have to be invoked.

It may turn out that deuterium was formed during the initial hot phase of the big bang or during subsequent epochs. In either case the abundance of this isotope provides an intriguing clue to evolution of the early Universe.

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Orthogonal mode emission in geometric models of pulsar polarisation

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The varying incidence of orthogonal 'states' of linear polarisation across the pulse of PSR 2016+28 is discussed in terms of a model with subpulse beams drifting round the pole of a rotating magnetic dipole.

THE position angle associated with the average linearly polarised radiation from many pulsars varies across the pulse in a manner consistent with a simple geometrical model first given by Radhakrishnan and Cooke^{1,2}. The model postulates polarised radiofrequency emission having properties character-

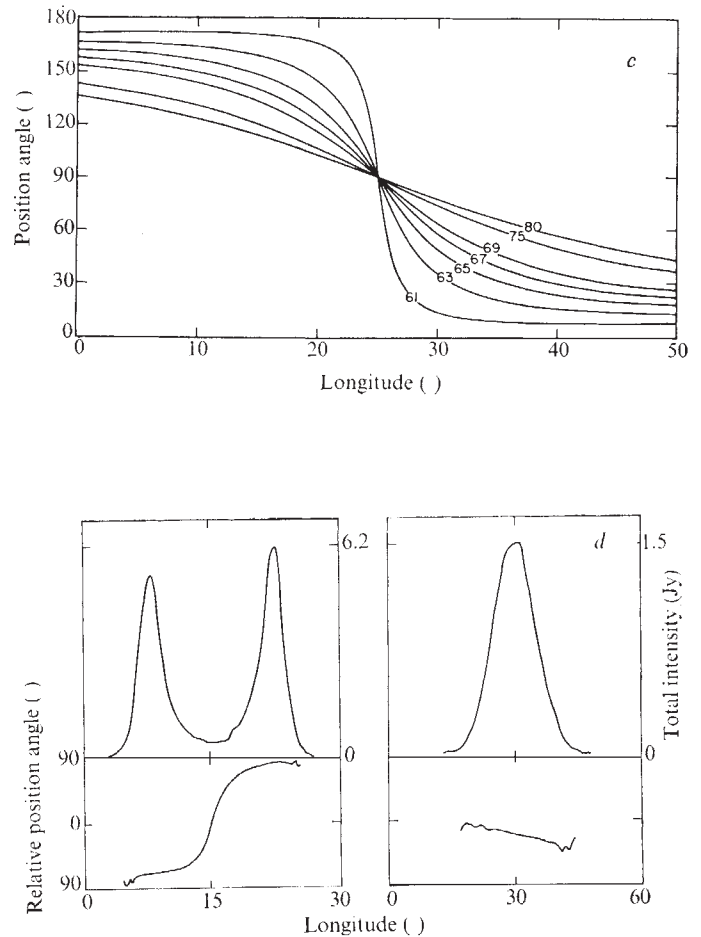
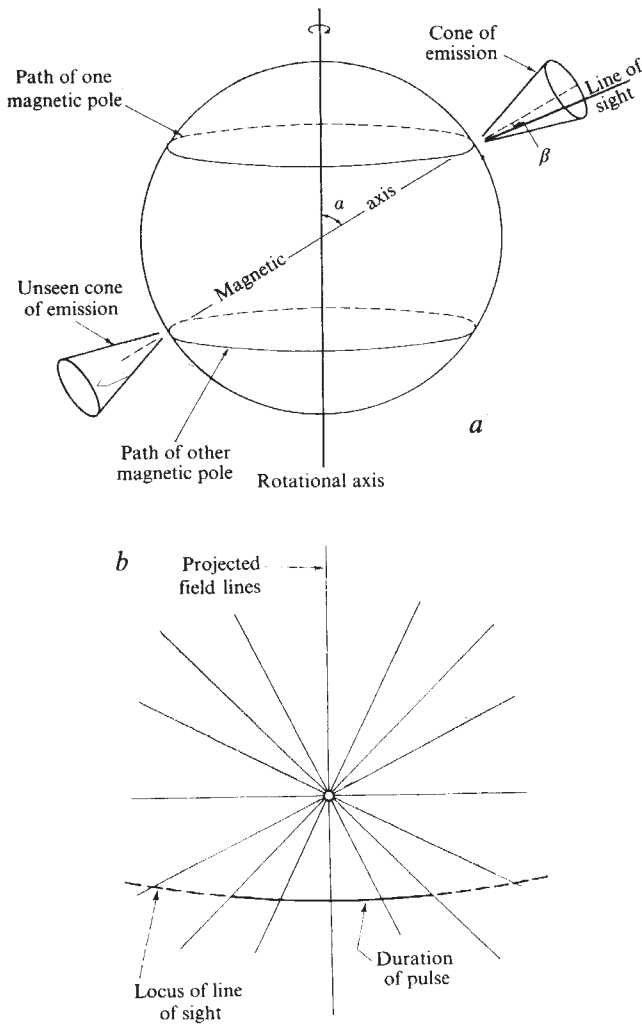
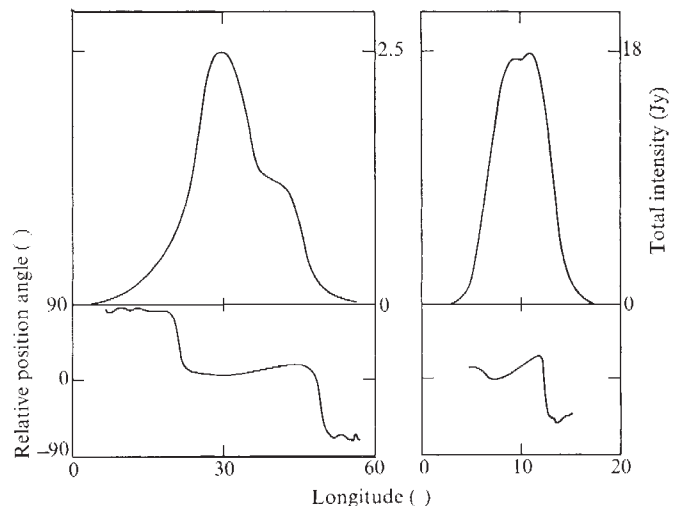


Fig. 1 *a*, Schematic representation of the Radhakrishnan and Cooke pulsar model¹. Emission is associated with the polar regions of an oblique, rotating, magnetic dipole. *b*, Field geometry near the pole of the star projected on to the celestial sphere. The radiofrequency radiation is expected to have polarisation characteristic of curvature radiation from particles accelerated along the field lines¹. *c*, Model waveforms of linear polarisation angle for a magnetic dipole axis inclined at 60° to the rotation axis; curves for observers at various polar angles are indicated. *d*, 430-MHz Arecibo observations of two pulsars (PSR 0525+21, left; PSR 1915+13, right) with linear polarisation angle morphologies which conform well with the Radhakrishnan-Cooke model angle waveforms in (*c*). The position angles are not absolute.

istic of the radiation from relativistic particles accelerated along diverging field lines near the poles of an oblique, rotating, magnetic dipole. Figure 1*a* shows the general configuration of the two emitting regions and Fig. 1*b* gives a view of the field lines as projected on to the celestial sphere. Position angles associated with the linearly polarised component of the radiation in this model are expected to coincide with the magnetic field projections in Fig. 1*b*. Thus the position angles are expected to progress monotonically through some portion of 180° during the pulse as the rotation of the star carries the polar cone of emission across the line of sight. Figure 1*c* gives model angle waveforms calculated for a dipole inclined at 60° and a range of observer inclinations; Fig. 1*d* presents observations of two objects as examples of the many pulsars which are nominally consistent with the model (see also, for instance, ref. 3).

There is, however, a second class of pulsars which displays more complicated polarisation angle behaviour that cannot be adequately accounted for on the basis of the above geometric model. In particular, there are several objects in the Arecibo pulsar polarisation survey⁴ which exhibit a rapid swing in the average polarisation angle by $\sim 90^\circ$ (for example, pulsars 1541+09, 1604-00, 1944+17, 2016+28 and 2020+28, see Fig. 2). Clearly the Radhakrishnan and Cooke model can account for only a single rapid change of $\sim 180^\circ$. Furthermore,

Fig. 2 430-MHz Arecibo observations of two pulsars which exhibit complex polarisation angle morphology (PSR 1944+17, left; PSR 2016+28, right). Rapid transitions of $\sim 90^\circ$ are evident in both cases. The position angles are not absolute.



Long.	$\langle I \rangle$	A1	A2	Polarisation angle (degrees)	
(degrees)	Jy	(degrees)		0	180
0.0	2.6	41	54	.211.1. 1 . 1111.	1
0.4	3.6	34	49	.22111. . . .1.	1
0.8	5.1	29	44	11221.	1
1.2	7.3	28	48	.12211.	1
1.5	10.4	28	41	.1221.	2
1.9	14.4	31	47	.1221.	3
2.3	19.7	31	48	.11211.	4
2.7	25.8	31	51	.11211.	4
3.1	33.2	32	55	.111111.	5
3.5	41.0	32	59	.11111.	6
3.9	49.7	29	62	.11111.	7
4.3	59.3	26	61	.11111.	7
4.6	67.5	25	61	.1121.	7
5.0	75.4	25	58	.1121.	7
5.4	81.9	25	56	.12211.	7
5.8	85.3	26	55	.12211.	8
6.2	87.5	27	54	.11211.	8
6.6	87.9	28	53	.11221.	8
7.0	87.7	30	55	.11221.	8
7.4	88.8	32	56	.1221.	8
7.7	89.3	33	56	.12211.	7
8.1	88.6	35	57	.11221.	8
8.5	89.2	36	61	.1221.	8
8.9	90.3	39	65	.1221.	8
9.3	90.6	41	70	.1211.	8
9.7	90.1	43	76	.1211.	8
10.1	88.3	43	84	.1111.	8
10.5	85.7	43	91	.1111.	8
10.8	81.9	39	95	.1111.	8
11.2	77.0	27	100	.1111.	8
11.6	70.6	178	107	.111.	8
12.0	62.9	169	110	.111.	8
12.4	54.9	173	113	.11.	7
12.8	44.9	166	114	.11.	7
13.2	34.8	161	117	.11.	6
13.6	26.1	156	123	.1.	5
13.9	19.6	156	128	.1.	4
14.3	14.9	156	134	.1.	4
14.7	11.7	158	136	.1.	3
15.1	9.3	158	136	.1.	2
15.5	7.6	160	138	.1.	2
15.9	6.2	161	133	.1.	1
16.3	5.2	165	126	.1.	1
16.6	4.4	165	124	.1.	1
17.0	3.7	166	121	.1.	1
17.4	3.0	163	117	.1.	1
17.8	2.4	164	131	.1.	1

Fig. 3 Probability distribution of linear polarisation angle at a sequence of longitudes across the pulse of pulsar 2016+28. Long. is the relative longitude in degrees, $\langle I \rangle$ is the amplitude of the total intensity average waveform in Jy, A1 is the vector-average position angle waveform in degrees (also plotted superposed on the distributions for comparison), and A2 is the corresponding arithmetic (amplitude independent)-average polarisation angle waveform. The display consists of probability densities of polarisation angle (in units of 10%) (with a decimal point indicating 0.05–5.0%) per resolution cell. The distributions reflect only those data which exceeded an amplitude threshold computed from the off-pulse noise baseline; the final column on the right gives the fraction of data above the noise threshold (in units of 10%).

90° rotation has been found to occur within subpulses of PSR 0809+74 (ref. 5), from subpulse to subpulse in PSR 2303+30 (ref. 4) (two objects dominated by drifting subpulses), and has been noted in several contexts recently⁶.

Preferred polarisation angles

Statistical studies of the Arecibo polarisation survey data⁷ indicate that most objects have, at a given longitude (360° longitude = 1 pulse period), two, nearly orthogonal, preferred polarisation angles of emission, and also that the

relative proportion of the two '90° states' often changes markedly across the pulse waveform (see also ref. 6). The varying proportion of the orthogonally-polarised constituents serves to distort a 'fundamental' angle waveform which, in most cases, is found to be consistent with the Radhakrishnan and Cooke model. (In other words, angle waveforms computed by averaging the data [90°] (instead of [180°]) would compare well ([90°]) with those in Fig. 1c.) Specifically, the characteristic bimodal nature of the position angle distribution arises, at least in part, from the rapid transition between states within

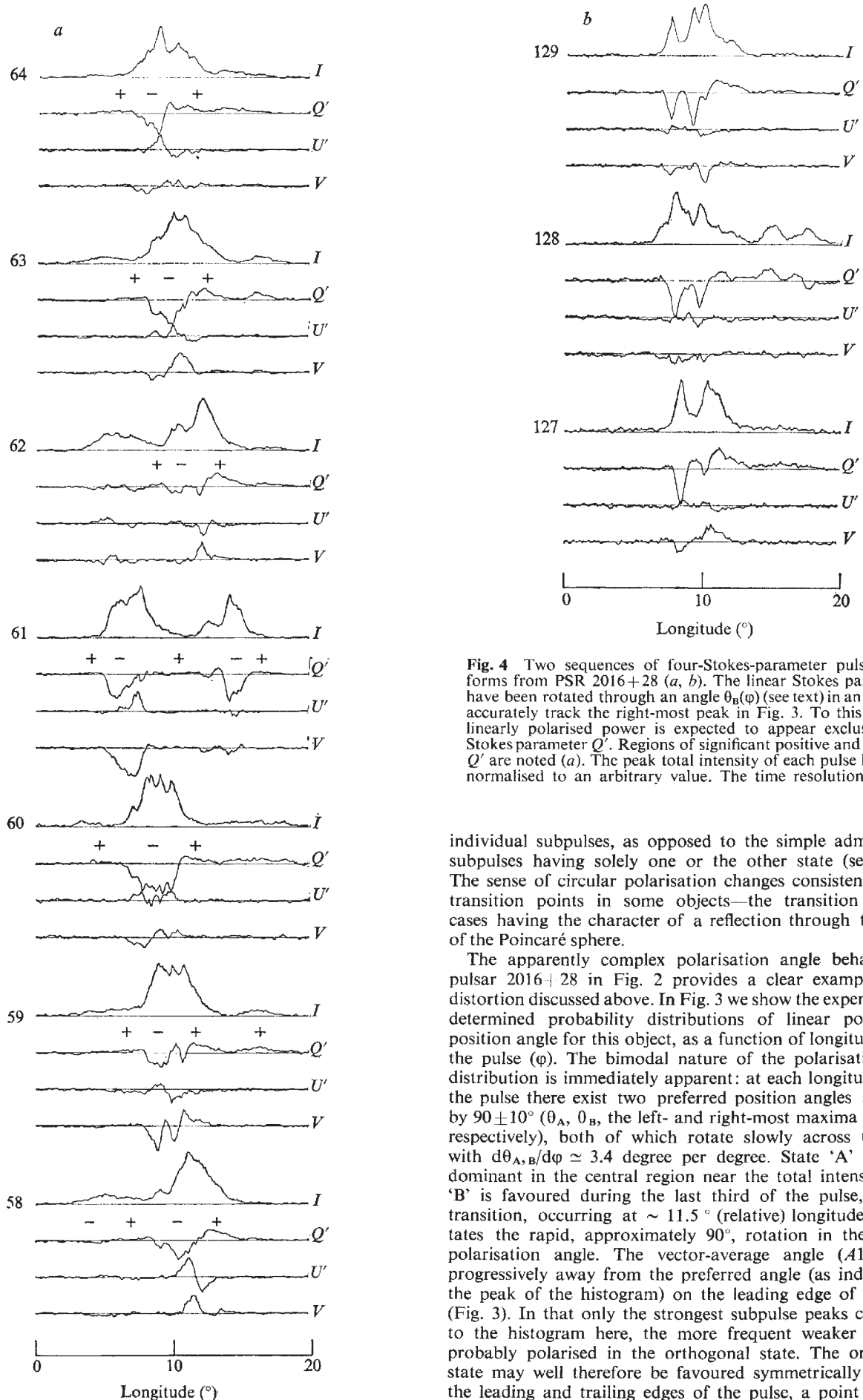


Fig. 4 Two sequences of four-Stokes-parameter pulse waveforms from PSR 2016+28 (*a*, *b*). The linear Stokes parameters have been rotated through an angle $\theta_B(\varphi)$ (see text) in an effort to accurately track the right-most peak in Fig. 3. To this end the linearly polarised power is expected to appear exclusively in Stokes parameter Q' . Regions of significant positive and negative Q' are noted (*a*). The peak total intensity of each pulse has been normalised to an arbitrary value. The time resolution is 0.5° .

individual subpulses, as opposed to the simple admixture of subpulses having solely one or the other state (see ref. 6). The sense of circular polarisation changes consistently at the transition points in some objects—the transition in these cases having the character of a reflection through the origin of the Poincaré sphere.

The apparently complex polarisation angle behaviour of pulsar 2016+28 in Fig. 2 provides a clear example of the distortion discussed above. In Fig. 3 we show the experimentally determined probability distributions of linear polarisation position angle for this object, as a function of longitude within the pulse (φ). The bimodal nature of the polarisation angle distribution is immediately apparent: at each longitude within the pulse there exist two preferred position angles separated by $90 \pm 10^\circ$ (θ_A , θ_B , the left- and right-most maxima in Fig. 3, respectively), both of which rotate slowly across the pulse with $d\theta_{A,B}/d\varphi \simeq 3.4$ degree per degree. State 'A' is clearly dominant in the central region near the total intensity peak, 'B' is favoured during the last third of the pulse, and the transition, occurring at $\sim 11.5^\circ$ (relative) longitude, precipitates the rapid, approximately 90° , rotation in the average polarisation angle. The vector-average angle ($A1$) moves progressively away from the preferred angle (as indicated by the peak of the histogram) on the leading edge of the pulse (Fig. 3). In that only the strongest subpulse peaks contribute to the histogram here, the more frequent weaker data are probably polarised in the orthogonal state. The orthogonal state may well therefore be favoured symmetrically on both the leading and trailing edges of the pulse, a point which is discussed further below.

Subpulse polarisation

Having investigated the occurrence of the two orthogonally-polarised angle states in the context of the average polarisation angle waveform, it is now clearly of interest to explore the nature of subpulse polarisation in the same light. Since the linear polarisation at any point in the pulse is observed to favour only two orthogonal position angles, the linear Stokes parameters at each point can be defined so that one linear component, say U' , is always zero and the other, Q' , reverses sign between the two states. This arrangement of the linear Stokes parameters (Q' , U') is effected for all longitudes ϕ by rotating the reference angle from position angle zero to θ_B (ϕ). Two sequences of pulses from PSR 2016+28, in all four Stokes parameters, I , Q' , U' and V , are shown in Fig. 4. From Fig. 4 it can be seen that, although the Stokes parameter U' is imperfectly nulled, the linearly polarised power preponderates in Q' and so our general view seems valid.

Models

We wish to interpret the Q' morphology in terms of the properties of subpulse beams according to the general tenets of the model proposed by Radhakrishnan and Cooke, whose views have been both extended and given a firm theoretical foundation by Sturrock⁸ and by Ruderman and Sutherland⁹. In addition, Ruderman¹⁰ explored the possibility that the drifting subpulse phenomenon is produced by narrow subpulse beams circulating round the dipole axis. The dimensions and motions of such subpulse beams seem to be consistent with a hollow-cone average beam—a concept suggested by Komesaroff² which now seems to be in agreement with much experimental evidence¹¹. Figure 5 summarises the various relevant features of these models in a single drawing where we have yet to speculate on the polarisation structure of the subpulse beams (indicated by $+/ -$ signs). Finally, the presence of two unresolved components in the total intensity average waveform of 2016+28 (evident in Fig. 2 as well as in other observations¹³) suggests that our line of sight passes somewhere near the inside edge of the cone as indicated in Fig. 5.

The Q' Stokes parameter

The Q' waveforms in Fig. 4 provide a tracing of the two orthogonal modes of emission (the sense of Q' distinguishing be-

tween the two) as the rotation of the star carries the pulsar's polar region across our line of sight. It is evident in Fig. 4a that central subpulses (those near the total intensity peak of the pulse) are most frequently characterised by a large negative excursion of Q' , bounded on one or both sides by smaller positive regions. The configuration depicted in Fig. 5 (*a, c, e*)—that is, a total power beam with a central lobe of one emission state ($Q' < 0$), bounded symmetrically by two sidelobes of the orthogonal state ($Q' > 0$)—seems to be the simplest time invariant beam largely compatible with the observed subpulse polarisation characteristics. In particular, the subpulse beams in Fig. 5 (*h, i*) do not reproduce the observations satisfactorily. Depending on the manner in which the line of sight cuts the subpulse beams, *a-e*, there is substantial flexibility in the kinds of subpulses produced: a well centred subpulse beam (Fig. 5c) produces a symmetrical, negative- Q' -dominated subpulse (Fig. 5C), whereas less central beams (Fig. 5a, e) result in increasingly asymmetrical, positive- Q' -dominated subpulses (Fig. 5A, B, D, E). Although it is difficult to identify positive- Q' -dominated subpulses at the extreme leading and trailing edges of the pulse (Fig. 4) because of the low intensity, Fig. 3 provides evidence that the orthogonal ($Q' > 0$) state was favoured symmetrically at the extremes of the pulse. That the symmetry is not more pronounced seems to indicate that we are able to see subpulse circulation further in the direction of Fig. 5a than in that of 5e. Furthermore, the subpulses of PSR 2016+28 generally seem to consist of one or more micropulses¹² which appear to perturb the foregoing picture, often producing a narrower version of the subpulse polarisation beam apparently superimposed on the broader beam. Some examples of subpulses dominated by micropulses can be seen in Fig. 4b. The actual nature of the radiation is doubtlessly much more complicated than we have outlined here and observations with much finer time resolution are required to study polarisation properties at the micropulse level.

Discussion

Although it is not yet clear whether every aspect of the polarisation properties of PSR 2016+28 are typical of pulsars, some generalisations can be made from the observations. Statistical studies similar to Fig. 3 show both that the two orthogonal '90° states' of emission are a characteristic feature of pulsar

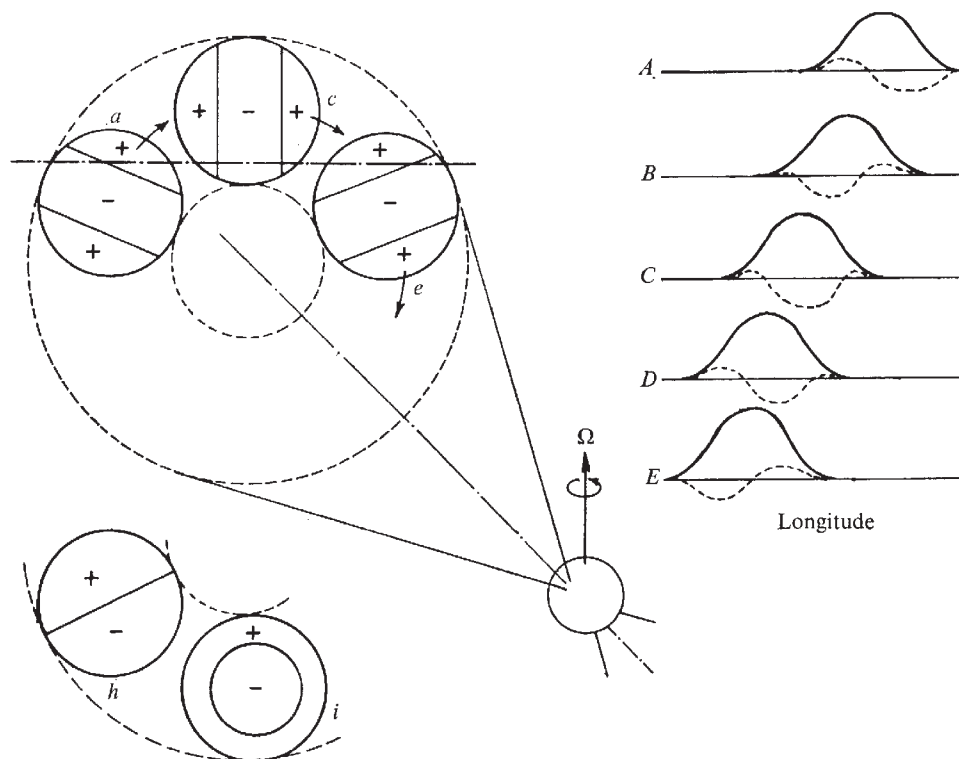


Fig. 5 A schematic drawing of the subpulse beam model for PSR 2016+28 under consideration (see text). This is essentially Ruderman's elaboration¹⁰ on the Radhakrishnan and Cooke model¹, wherein subpulse beams are thought to circulate within a hollow-cone average beam, except that we now specifically consider the nature of subpulse polarisation ($+/ -$ indicates the two orthogonal lobes of subpulse polarisation). The dashed curve traces the approximate path of the line of sight across the polar emission region. Sketches of subpulse waveforms corresponding to cuts through three of the illustrated subpulse beams (*a, c, e*) are given on the right (*A, C, E*), as well as two intermediate cases (*B, D*). Polarised subpulse lobe structures (*h*) and (*i*) do not seem to correspond to the observations.

radiation and that a much larger group of pulsars can now be well described by a simple geometric model when the nature of their radiation is appropriately considered. The polarised individual pulse waveforms of PSR 2016+28, as well as other objects with strong drifting subpulse modulation, have a straightforward interpretation wherein subpulse beams with a rigid polarisation pattern are viewed in different orientations as time progresses. Pulsars 1919+21, 1944+17, 2016+28, the second component of 2020+28 and 2303+30 in the Arecibo survey as well as PSRs 0031-07 and 0809+74 in other studies⁶ all bear out various features of this view. On the basis of current pulsar models we have suggested one method of producing subpulse beams which change their orientation on the sky as required: narrow subpulse beams with time-invariant polarisation patterns circulate around the dipole axis maintaining a fixed orientation to the plane of the magnetic field. Our speculations regarding the polarised lobe structure of the subpulse beams are less certain but may have some generality. Neverthe-

less, we hope that they will serve to prompt some further theoretical investigations of emission mechanisms and propagation effects¹³ in pulsar magnetospheres.

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Structure of locust adipokinetic hormone, a neurohormone that regulates lipid utilisation during flight

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Adipokinetic hormone, isolated from locust corpora cardiaca, has been identified as a blocked peptide: PCA-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH₂. The detailed structure is based on mass spectrometric data, substantiated in part by dansyl-Edman and carboxypeptidase data on thermolytic fragments. This is the first peptide hormone from an insect neuroendocrine organ to be fully characterised.

In insects, it has been shown (for reviews, see refs 1 and 2) that peptide and protein hormones are produced by neurosecretory cells or glandular cells associated with the neuroendocrine system. These neurosecretory cells are in the brain, the corpora cardiaca and the medial nervous system. Much work has been carried out investigating the factors present in the corpora cardiaca, which are neurohaemal organs that both store and release neurosecretory products synthesised in the brain, and contain intrinsic glandular cells producing their own secretions. So far, factors that affect cuticle tanning³, water balance⁴, carbohydrate metabolism⁵⁻⁷, muscle contraction⁸⁻¹⁰ and lipid metabolism¹¹⁻¹³ have all been found in the corpora cardiaca from various insects, and many of these factors have been shown to be of a peptide nature. Until now, however, none of these active substances has been purified and fully characterised. We report here the purification and identification of one of these factors, the adipokinetic hormone from the locust.

The existence of an adipokinetic hormone in locusts, located in the corpora cardiaca and concerned with flight activity, was first suggested by Mayer and Candy¹⁴ and

Beenackers¹⁵. During the initial stages of flight, before release of the hormone, energy is derived from trehalose in the haemolymph¹⁴. A few minutes after the commencement of flight, however, the hormone is released from the intrinsic secretory cells located in the glandular lobes of the corpora cardiaca¹³ into the haemolymph; this hormone has two separate sites of action. It causes the release of specific diglycerides from the fat body^{15,16} into the haemolymph. These diglycerides, which are transported as lipoproteins in the haemolymph¹⁷, are used by the flight muscle as a source of energy. The adipokinetic hormone also stimulates the utilisation of these lipids by the flight muscle¹⁸. Consequently, the hormone is important in the maintenance of prolonged flight activity, by enabling the locust to utilise its lipid stores for energy. Mayer and Candy¹⁴ first indicated that adipokinetic hormone was a peptide as its biological activity was destroyed by proteolytic enzymes.

Isolation

Throughout the purification procedure the hormone was located by measuring its lipid-mobilising activity when injected into adult male *Locusta*. Samples to be injected were dissolved in 20 μ l simple insect saline (7.5 g NaCl + 0.375 g KCl l⁻¹). Aliquots (5 μ l) of haemolymph were withdrawn immediately before and 1 h after injection of the samples and the changes in haemolymph lipid content measured as described previously¹³. A unit of adipokinetic activity was arbitrarily defined as the quantity of hormone that causes the release of 1 μ g lipid per μ l haemolymph in 1 h.

The hormone was isolated from the glandular lobes of the corpora cardiaca from both the migratory locust, *Locusta migratoria*, and the desert locust, *Schistocerca gregaria*. The material extracted from 3,000 corpora cardiaca (750 μ g)

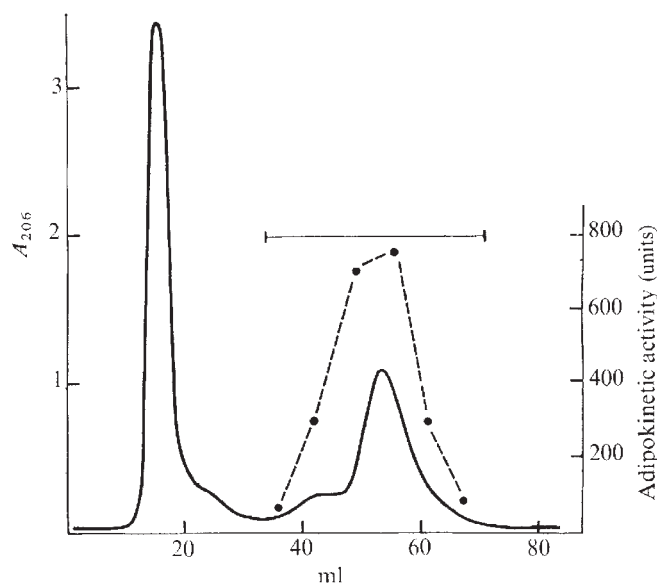


Fig. 1 Separation of an extract of locust corpora cardiaca on G-75-50 glass beads. The residue from a methanol extract of the glandular lobes from 500 locusts was dissolved in 1 ml distilled water and applied to a column (2×25 cm), equilibrated and eluted with water. Flow rate 30 ml h^{-1} . The effluent was collected in 2-ml fractions. The adipokinetic activity of every third fraction, diluted 1:49 with simple saline was measured, taking the mean response from 4 test insects. —, A_{206} ; ●, adipokinetic activity; ———, pooled fractions.

has been used to characterise the hormone fully. The glandular lobes were dissected out under simple saline and placed in methanol. The tissue was disrupted using an MSE 100W ultrasonic disintegrator, and the insoluble debris removed by centrifugation. Methanol was evaporated from the supernatant and the residue redissolved in distilled water. This aqueous extract was applied to a controlled-pore glass bead column (G-75-50 beads, Sigma) and the column eluted with distilled water. A profile as shown in Fig. 1 was obtained. The hormone was eluted as a single peak at an elution volume slightly less than the total column volume. The active fractions were pooled and water was removed under reduced pressure at 50°C . The residue was dissolved in methanol and applied to a silica gel thin-layer plate prewashed with methanol. The chromatogram was developed in isopropanol-water-acetic acid (25:10:1 v/v) which separated out an ultraviolet-absorbing area of R_f 0.67 that contained the adipokinetic activity. The active material was eluted into methanol and subsequently shown to be the pure hormone. The purification procedure, showing the recoveries of active material at each stage, is summarised in Table 1. Overall, approximately half of the original adipokinetic activity in the glandular lobes was recovered by this method.

Between 100 and 250 pmol (calculated from the amino acid content) of the pure hormone were obtained from one *Locusta* compared with 200–250 pmol from one *Schistocerca*. Taking into account the losses during purification it is estimated that the glandular lobes from the corpora cardiaca of one adult *Locusta* contain 0.25–0.55 μg stored hormone, whereas each adult *Schistocerca* contains 0.50–0.80 μg .

Structure elucidation

Analysis of the hormone after acid hydrolysis gave the amino acid composition shown in Table 2a and suggested it was a nonapeptide. The amino acid compositions of the peptides isolated from both *Locusta migratoria* and *Schistocerca gregaria* were found to be almost identical. The hormone did not react with dansyl-chloride¹⁹ and in electro-

Table 1 Purification of adipokinetic hormone from glandular lobes of locust corpora cardiaca

Fraction	Total adipokinetic activity (units)	s.e. of estimate (%)	Recovery (%)
Methanol extract	1,158,300	14	100
Pooled column fractions	982,800	5	85
Thin-layer chromatography fraction, R_f 0.67	649,300	12	56

Values are for a preparation from the glandular lobes of 500 locusts.

phoresis at 50 V cm^{-1} in formate buffer (pH 2.1) on Whatman number 1 paper, it moved with the neutral marker, 1-diaminonaphthalene-sulphonic acid (DNS-OH). It also coelectrophoresed with the neutral marker alanine at pH 6.5 (pyridine-acetate buffer), the peptide being located in each instance by staining with starch-iodide after chlorination²⁰. These results suggest that the peptide does not possess any free amino or carboxyl groups.

The blocked N-terminal amino group of the hormone prevented direct determination of the sequence using the dansyl-Edman procedure¹⁸. Enzymatic digestion of the peptide with thermolysin for 4 h at 37°C (120 nmol hormone, 1.2 nmol thermolysin in $500 \mu\text{l}$ $0.067 \text{ M NH}_4\text{HCO}_3$ containing $2.5 \mu\text{mol CaCl}_2$), caused the appearance of a residue with a free amino group, identified as phenylalanine using the dansyl-chloride procedure. Electrophoresis of the digest at 50 V cm^{-1} on Whatman 3MM paper at pH 6.5 followed by staining, showed two fragments—one acidic with a mobility of $+0.55$, and one basic with a mobility of -0.29 , relative to aspartic acid. Electrophoresis at 50 V cm^{-1} on Whatman number 1 paper at pH 2.1, also indicated the presence of two fragments, one which moved with the neutral marker, DNS-OH, and the other with a mobility of $+0.37$ relative to dansyl-arginine. Crude molecular weight determinations from these electrophoretic mobilities^{21,22} indicated a value of approximately 350 for the acidic (N-terminal) fragment and 700–800 for the basic (C-terminal) fragment. Elution of these peptides from the electrophoretogram gave an 80% yield of the N-terminal fragment and 20% of the C-terminal fragment. The amino acid compositions of the fragments after acid hydrolysis are shown in Table 2b and c.

The N-terminal fragment was degraded sequentially from its C-terminal end by enzymatic digestion, and the digests analysed on the amino acid analyser without previous hydrolysis. Incubation of 9 nmol of this fragment with penicillocarboxypeptidase-SI (ref. 23) for 3.5 h at 37°C (in $100 \mu\text{l}$ 0.2 M pyridine-acetate, pH 4.2, containing 0.15 nmol penicillocarboxypeptide-SI) released 8.7 nmol of a ninhydrin-positive substance, identified as asparagine using the dansyl-chloride procedure. Incubation of 8 nmol of the fragment

Table 2 Amino acid composition after acid hydrolysis of adipokinetic hormone and fragments obtained by thermolysin digestion

Amino acid	Hormone	Relative molar quantity in:	
		N-terminal fragment	C-terminal fragment
Asp	1.82	1.07	1.15
Thr	1.82		1.71
Glu	1.11	1.49	
Pro	0.96		1.00
Gly	1.05		1.71
Leu	1.00	1.00	
Phe	0.97		0.77

Hydrolyses were carried out in 6 M HCl containing 0.1% phenol. Results are expressed relative to Leu = 1.00 for the hormone and N-terminal fragment, and relative to Pro = 1.00 for the C-terminal fragment.

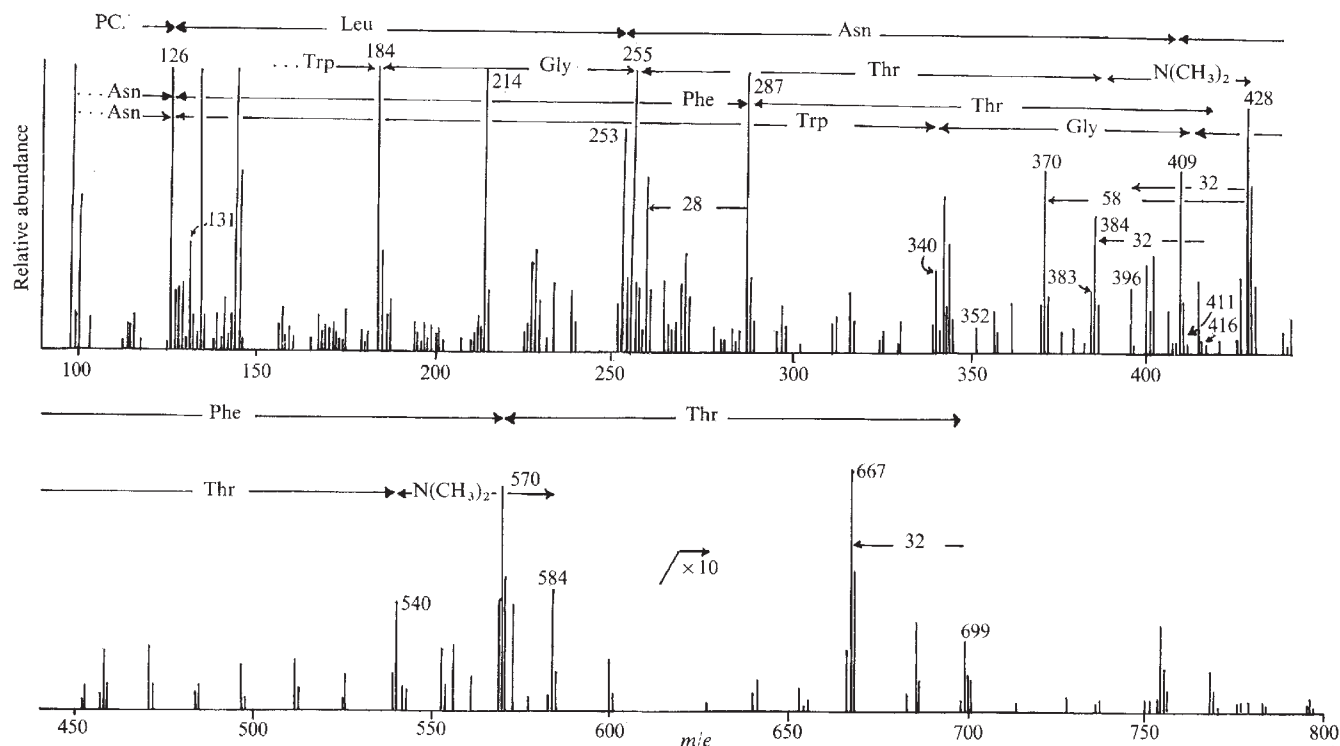


Fig. 2 Mass spectrum at a source temperature of 250 °C of a permethyl derivative of adipokinetic hormone. . . . Asn, . . . Phe and . . . Trp refer to an N-C cleavage fragmentation pathway^{25, 29}.

with (DFP)-treated carboxypeptidase A (prepared by the second procedure of Ambler²⁴) for 24 h at 37 °C (in 25 μ l 0.2 M NH_4HCO_3 containing 80 pmol carboxypeptidase A) released 3.8 nmol asparagine and 0.8 nmol leucine. This shows that the fragment has the sequence -Leu-Asn at its C-terminal end, leaving glutamic acid as the N-terminal residue. The N-terminal amino group of this residue is blocked in some way, as it does not react with dansyl-chloride.

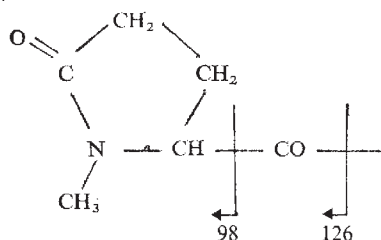
The amino acid sequence of the C-terminal fragment was first investigated using the dansyl-Edman degradation procedure. This indicated the sequence Phe-Thr-Pro-Asn- for the first four residues.

So far a partial structure for adipokinetic hormone could be written as: X-Glx-Leu-Asn - - - Phe-Thr-Pro-Asn-, where X represents some blocking group and the dashed line a probable linkage between fragments. An additional threonine and a glycine residue present in the amino acid analyses of both the intact hormone and the C-terminal thermolytic fragment remained unaccounted for.

Mass spectrometric methods²⁵ were applied both to the thermolytic fragments and to the intact hormone to determine its overall structure.

Blocked N terminus

Examination of the acetyl-permethyl derivative²⁶ of a thermolytic digest of adipokinetic hormone was made using the mixture analysis fractional distillation technique^{27, 28}. Low temperature scans showed an N-terminal sequence fragment at m/e 98 which together with a weak signal at m/e 126 is characteristic of an N-terminal pyrrolidone acid residue.



When the associated signal m/e 126 is weak as here, this shows conclusively²⁵ that the N-terminal residue is a pyrrolidone carboxylic acid (PCA) residue before derivative formation. After these signals, a sequence PCA-Leu was established using a signal at m/e 253.

At higher source temperatures a second component volatilised giving a mass spectrum with signals at m/e 204, N-terminal Phe; at 333, Phe-Thr; at 430, Phe-Thr-Pro; and at 586, Phe-Thr-Pro-Asn.

Other signals in the spectrum provided firm evidence of the presence of an additional amino acid residue, other than those in the amino acid analysis of the acid hydrolysate of adipokinetic hormone (Table 2).

Complete structure and the presence of tryptophan

The mass spectrum described above and all spectra of the intact hormone derivatives (see below) contain strong signals at m/e 144, 184, 214 or 215 (refs 25 and 29), (or the signals of their deuterated equivalents), together with sequence ions, which imply the presence of tryptophan in adipokinetic hormone. Although unexpected, it could readily be placed in the sequence by analysis of the 'thermolytic' and 'intact hormone' spectra. For example, the spectrum described above, interpreted as Phe-Thr-Pro-Asn, contained further signals showing that the asparagine is followed in sequence by tryptophan. In one of the thermolysin digests examined, this tryptophan was C-terminal (m/e 215), although it was not in the studies of the undigested hormone as shown below. The presence of this tryptophan was confirmed and the sequence was extended to the true C terminus by analysis of the spectra of the intact hormone derivatives.

Figure 2 shows the mass spectrum of the acetyl-permethyl derivative²⁶ of intact adipokinetic hormone, and was interpreted as follows: Abundant sequence ions at m/e 98, 253, 409, 570 and 699 are readily assigned to PCA-Leu-Asn-Phe-Thr as the N-terminal sequence, confirming the blocked N terminus and the presumptive Asn-Phe bond assumed from the thermolysin data. Other major

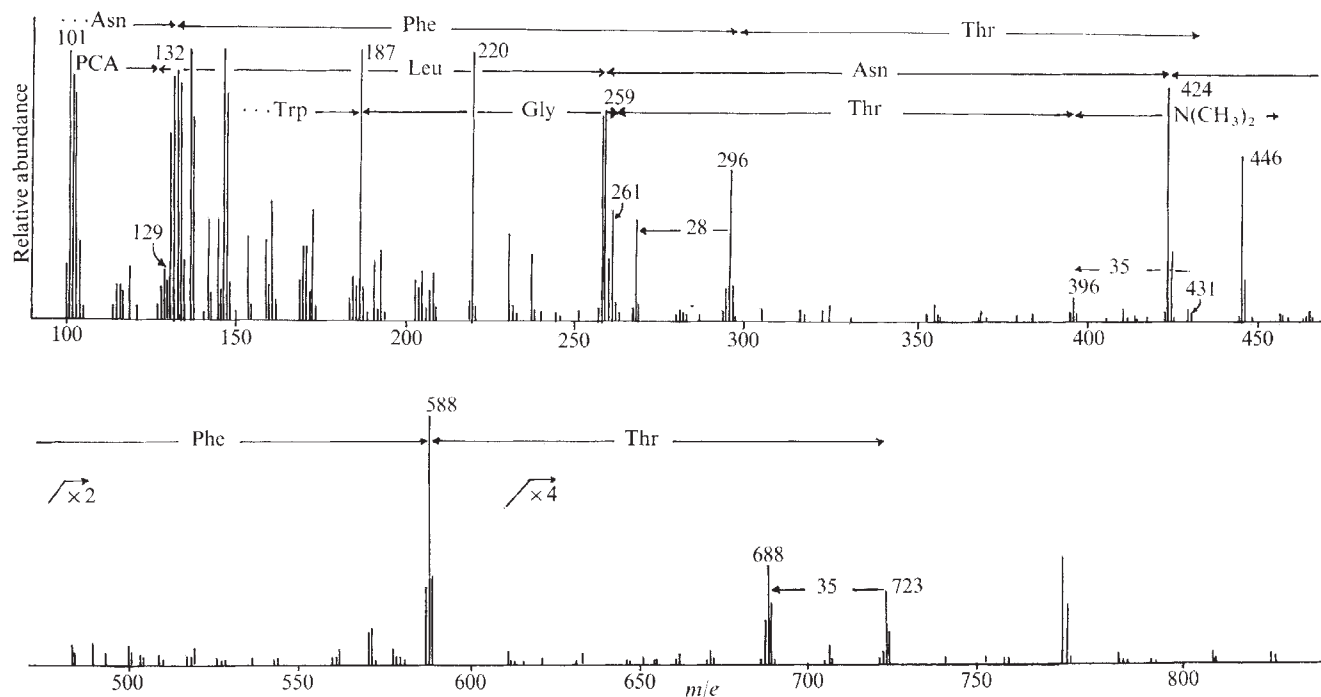
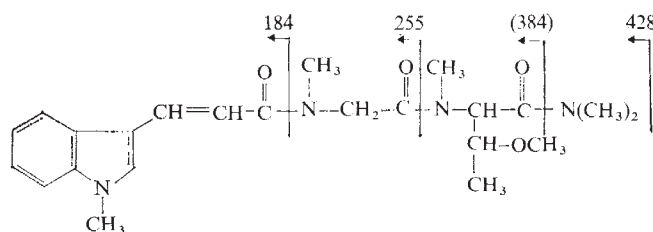


Fig. 3 Mass spectrum at a source temperature of 270 °C of a perdeuteromethyl derivative of adipokinetic hormone. Mass shifts are correlated with signals in Fig. 2 to support the assignments made. For details see text.

signals in the spectrum derive from N–C cleavage pathways^{25,27} which originate from signals at m/e 126 (asparagine), 131 (phenylalanine) and 184 (tryptophan).

The most important of these N–C cleavage pathways, as regards interpretation of structure, is that emanating from tryptophan at m/e 184. Testing for amino acid mass differences from m/e 184 the first major unassigned signal is at m/e 255, a glycine mass difference away. That this signal could be due to a threonine residue eliminating its side chain with rearrangement, is unlikely, as neither a threonine sequence ion (m/e 313) nor an associated loss of methanol (m/e 281) are present in the spectrum. Moving from m/e 255 to higher mass, m/e 384 can be assigned to a threonine mass difference, losing the expected 32 mass units (CH_3OH) to give the smaller signal at m/e 352. The reason for m/e 370 not being assigned to a serine residue is that no expected loss of 32 from this signal is observed. After m/e 384 a 44 mass unit difference to m/e 428 is assigned as a C-terminal amide in adipokinetic hormone (an ester would have given a signal at m/e 415). The assignment of m/e 428 to the structure shown below is fully substantiated by the expected losses of methanol (m/e 396) and the threonine side chain (m/e 370).



Probably the most powerful confirmation of low resolution structure assignments in mass spectrometry is made by observing the shift in signals associated with the synthesis of deuterated derivatives³⁰. The mass spectrum of perdeuteromethyl adipokinetic hormone is shown in Fig. 3. Although the overall appearance of the spectrum is slightly changed by temperature and derivative procedure effects, all the major assignments made in Fig. 2 are vindicated by

the correct mass shift of the corresponding signals. In particular, m/e 187, 261 and 446 in Fig. 3 have moved by 3, 6 and 18 mass units, as would be expected from the structure shown above, and justify the assignment of C-terminal Trp–Gly–Thr–amide. The presence of tryptophan in adipokinetic hormone has since been confirmed both after acid hydrolysis in the presence of 2% thioglycolic acid³¹ (30% tryptophan recovery), and after hydrolysis in *p*-toluene sulphonic acid³² (50% recovery). Summarising the fragmentation data of Figs 2 and 3 the overall structure of adipokinetic hormone is shown to be: PCA–Leu–Asn–Phe–Thr–Pro–Asn–Trp–Gly–Thr–NH₂. The hormone is therefore a blocked decapeptide and its molecular weight is 1,158.

Biological activity

The biological activity of the fragments produced by thermolysin digestion of the hormone was investigated and compared with the activity of the natural hormone. Figure 4 shows a dose–response curve for pure adipokinetic hormone injected into adult male *Locusta*. At doses above about 5 pmol of hormone the response is near maximal. Doses of the thermolytic digest containing up to 30 pmol of each fragment, however, did not elicit any lipid-mobilising response when injected into locusts. (Thermolysin was inactivated by boiling before injection of the sample.) Consequently, it seems that cleavage of the hormone between its third and fourth residues completely destroys its biological potency, as neither of the resulting fragments possesses any adipokinetic activity.

It is clear that biologically active peptides are components of various control in insects. It was not until relatively recently, however, that any such peptide had been isolated in sufficient quantity for its molecular structure to be determined. The amino acid sequences of a 27-residue peptide from the male fruit fly's accessory gland that influences female mating behaviour³³, and of a pentapeptide that is a proposed neurotransmitter in the cockroach³⁴, have both been elucidated. To our knowledge the data presented here form the first instance of the complete characterisation of an insect neurohormone. This elucidation of the detailed structure of an insect peptide hormone may be the first step in development of new ways to control

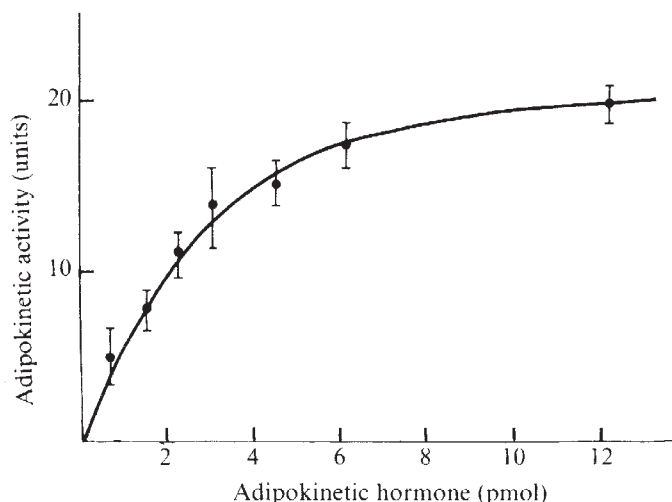


Fig. 4 Dose-response curve for locust adipokinetic hormone. Samples of the pure hormone, dissolved in 20 μ l simple saline, were injected into 16-d-old adult male *Locusta*. Each point represents the mean activity $\pm$ s.e. from 6 animals.

insect pests. Once the structure-activity relationships are resolved, it may be possible to design compounds which interfere specifically with the normal functioning of the insect neurosecretory system; such interference will disrupt many morphological, metabolic and physiological events. Indeed it has been shown that treatment with conventional insecticides provokes the release of large quantities of neurohormones, and it is suggested that such release may be the, or a contributory, cause of death^{35,36}.

The cerebral neurosecretory cell-corpus cardiacum complex in insects is analogous with the hypothalamic-neurohypophyseal system in vertebrates³⁷. Therefore it is of particular interest that the insect hormone identified here possesses features in common with certain hormones secreted by the hypothalamus. Thus both the N-terminal pyroglutamic acid residue and the C-terminal amide shown for adipokinetic hormone in the present paper, are also seen in thyrotropin-releasing hormone^{38,39} and luteinising hormone-releasing hormone^{40,41}. A recently characterised crustacean neurohormone, the red-pigment-concentrating hormone from shrimps⁴², also possesses these features. Moreover, in spite of its different biological function this shrimp hormone has a similar amino acid sequence to adipokinetic hormone (adipokinetic hormone: PCA-Leu-Asn-Phe-Thr-Pro-Asn-Trp-Gly-Thr-NH₂; shrimp hormone: PCA-I-leu-Asn-Phe-Ser-Pro-Gly-Trp-NH₂). Some

early experiments^{43,44} in which extracts of insect corpora cardiaca caused a bleaching effect when injected into shrimps emphasises the similarity in structure of these two hormones. Further characterisation of hormones is clearly necessary before it can be ascertained whether close structural relationships exist among all arthropod neurohormones.

The synthesis of adipokinetic hormone is in progress. It is expected that the synthetic peptide will confirm the structure of the hormone postulated here.

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3' Non-coding region sequences in eukaryotic messenger RNA

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The sequence A-A-U-A-A-A is present in six different purified messenger RNA molecules (specifically the α - and β -globulin mRNAs of rabbit and human, the immunoglobulin light chain mRNA of mouse (MOPC 21) and the ovalbumin mRNA of chicken) about 20 residues away from the 3'-terminal poly(A) sequence. In addition, a large selection of the 3' non-coding regions of rabbit and human globin mRNAs (both the α - and β -globin mRNAs) are 85% homologous, demonstrating that this region is significantly conserved in evolution.

EUKARYOTIC mRNAs have been shown to contain several surprising structural features. For instance, they contain modified terminal sequences, specifically the 3' terminal poly(A)¹ and the 5' terminal m⁷Gppp or cap structure^{2,3}, both of which are added to mRNA post-transcriptionally. In addition, mRNA from higher organisms has been shown to contain substantially more sequence than is required to code for protein. A generalised diagram of the organisation of the various regions in mRNA is shown in Fig. 1.

The cap structure and at least a part of the 5' non-coding sequence are thought to be involved in the binding of the mRNA to the ribosome in the correct initiation of protein

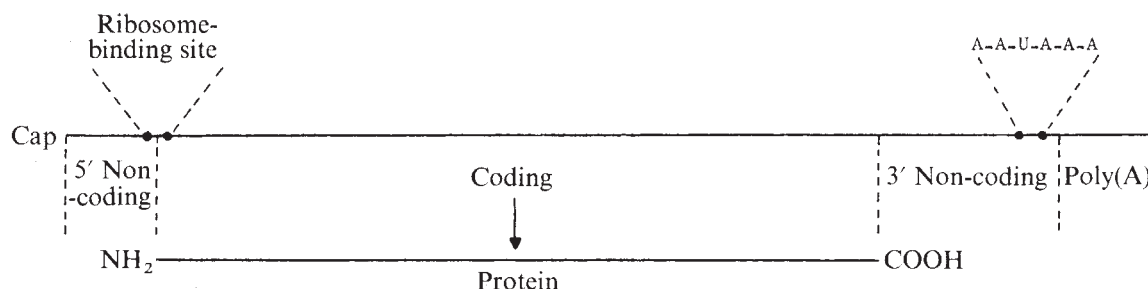


Fig. 1 Generalised diagram of an mRNA molecule. There are, however, exceptions to this general arrangement. For example, histone mRNAs lack poly(A) and polio mRNA lacks Cap⁴. See text for explanation of ribosome binding site and A-A-U-A-A-A.

synthesis⁴. The details of these processes are, however, still unknown. Thus a region of about ten nucleotides, referred to as the 'ribosome-binding site' (Fig. 1) seems to base-pair with a sequence at the 3' end of the 16S ribosomal RNA in *Escherichia coli*^{5,6}. Although such a process has yet to be demonstrated in higher organisms, it would seem likely that such an important mechanism would be conserved in evolution.

In contrast to the 5' non-coding region, the function of at least a part of which the sequence is known, there is no direct information on the function of the 3' non-coding region. This is surprising as the 3' non-coding region is longer than the 5' non-coding region—at least with the globin mRNAs, for which reasonably accurate estimates have been made⁷. To find out more about this part of the structure of mRNA we have developed a method that has enabled us to determine the sequence of that part of the 3' non-coding sequence which lies immediately adjacent to the poly(A)^{8,9}. By comparing this sequence from a number of different mRNAs we have attempted to locate common features which might indicate regions with particularly important functions. We therefore present here sequences of varying lengths adjacent to the poly(A) of rabbit α and β globins^{7,9}, human α and β globins (N.J.P. and J. I. Langley, unpublished), mouse immunoglobulin light chain¹⁰ and chicken ovalbumin¹¹ mRNAs. A comparison of particular interest was that of the sequences of the globin mRNAs in rabbit and man. If these 3' non-coding sequences showed reason-

able evolutionary stability this would argue for their importance functionally.

Sequence homologies

Figure 2 shows the sequences adjacent to poly(A) for the six mRNAs, specifically rabbit α and β globins, human α and β globins, mouse immunoglobulin light chain (MOPC 21) and chicken ovalbumin mRNA. All six mRNAs possess the homologous sequences A-A-U-A-A-A (enclosed in boxes) between 14 and 20 nucleotides from the poly(A) sequence. In addition, human β -globin mRNA and chicken ovalbumin mRNA have a more extensive region of sequence (Fig. 3). In detail a region of 13 nucleotides is completely homologous, save one nucleotide, for the two mRNAs. It is also interesting, although possibly less significant, that the other four mRNA sequences have an additional G residue conserved on the 3' side of the homologous sequence: A-A-U-A-A-A-G (Fig. 2).

Another region of partial sequence homology between the six mRNAs is the sequence directly adjacent to the poly(A). Thus five of the six mRNAs possess the sequence G-C-poly(A). The immunoglobulin light chain mRNA, however, does not possess this sequence. This result is a correction to a previous publication¹⁰ in which a single G residue was incorrectly inserted. Thus mRNA can have different sequences adjacent to poly(A), a result in agreement with other studies^{16,17}.

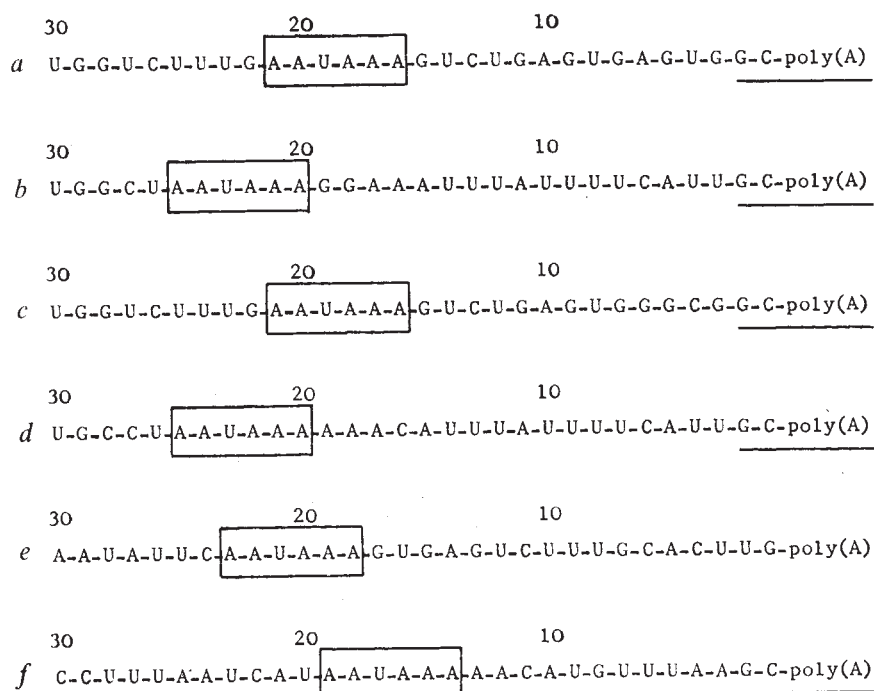


Fig. 2 Comparison of sequences adjacent to poly(A) in six eukaryotic mRNAs: a, rabbit α -globin; b, rabbit β -globin; c, human α -globin; d, human β -globin; e, mouse immunoglobulin light chain; f, chicken ovalbumin mRNAs. The full sequences of the globin mRNAs are shown in Fig. 4, whereas those of the immunoglobulin light chain and chicken ovalbumin mRNAs are presented elsewhere^{10,11}. Boxed sequences denote homology, whereas underlined sequences denote partial homology. Numbers indicate distance from poly(A). The sequence analysis of the six mRNAs is described in detail elsewhere (refs 7, 10 and 11, and N.J.P. and J. I. Langley, unpublished). Each mRNA was copied into ³²P-labelled cDNA using DNA polymerase I (*E. coli*) in the presence of Mn²⁺ and α -³²P-labelled deoxyribonucleoside triphosphates with oligo(dT)₁₀ as primer⁸. In such conditions, short transcripts of the mRNAs were obtained (50–100 nucleotides long) complementary to the 3'-terminal regions of the mRNAs⁷. These cDNA molecules were then digested with endonuclease IV (refs 12 and 13) and the products obtained were sequenced using standard DNA sequencing procedures^{13–15}. Note that the mouse immunoglobulin light chain and the rabbit α - and β -globin sequences contain corrections to previously reported sequences^{9,10}. These mistakes were detected by improvements in the sequencing methodology^{7,11}.

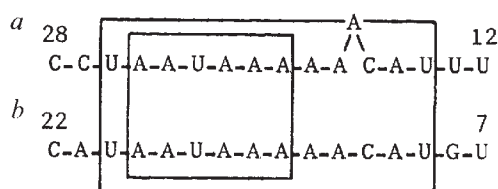


Fig. 3 Comparison of a small section of human β globin (*a*) and chicken ovalbumin mRNAs (*b*). Outside box encloses homologous sequence, inside box encloses sequence common to all six mRNAs. Numbers denote distance of that nucleotide from poly(A) sequence.

Comparison of human and rabbit sequences

One direct test as to the functional importance of a particular nucleotide sequence is to establish how conserved or otherwise that sequence is among different species. If the sequence has completely diverged as in the case of satellite DNA¹⁸ its function may be regarded as not sequence specific. Alternatively if the sequence has been very highly conserved, its function can be considered to be highly specific, and central to the overall biological role of the molecule. Such may be the case with the 3' non-coding regions of globin mRNA. Figure 3 compares the nucleotide sequences determined for our own and other studies for rabbit α - and β -globin and human α - and β -globin mRNAs. As indicated, the sequences of these 3' non-coding regions are not yet completely known as there is a gap of about 50 nucleotides in both α -globin mRNA sequences and another of 50 or more nucleotides in the β -globin mRNA sequence⁷. Nevertheless, sufficient of the sequence is known to make a preliminary comparison of the data informative.

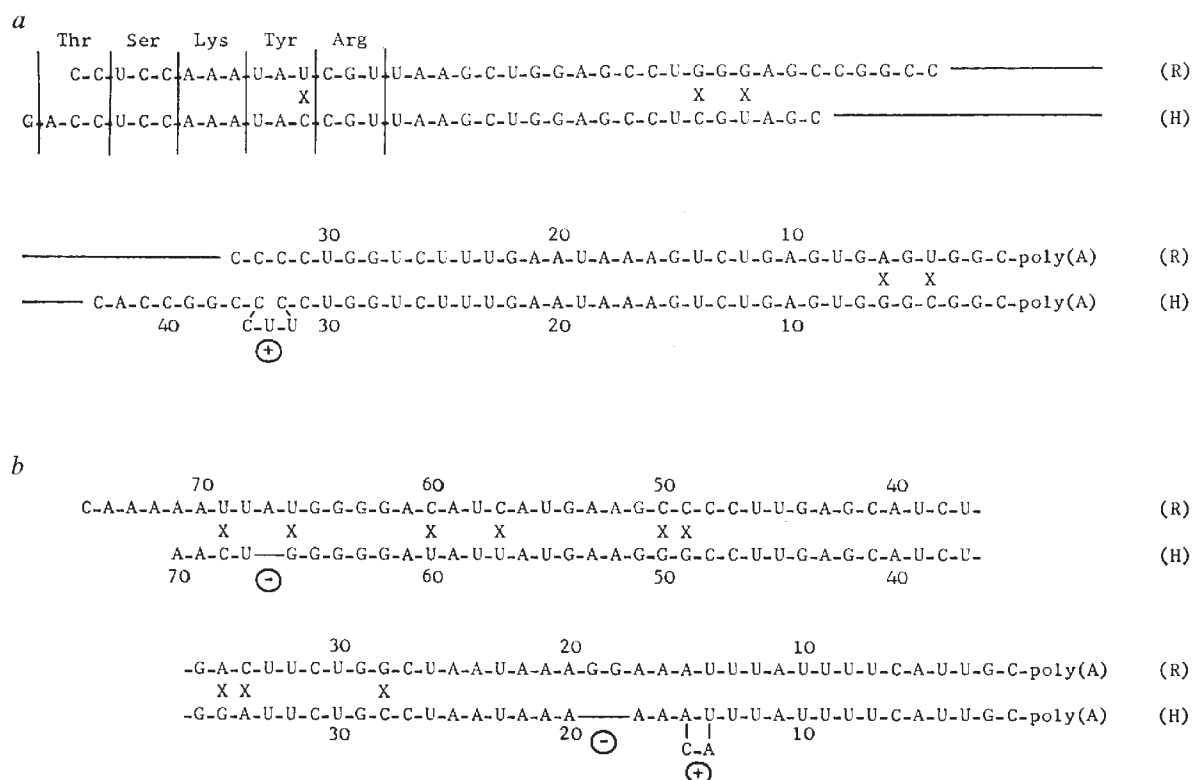
Both the α - and β -globin mRNA sequences are extensively conserved between human and rabbit. Indeed, of

129 nucleotides from the 3' non-coding sequences, only 11 substitutions occur. In addition, there are four examples of deletions and insertions of between one and three nucleotides. This may be regarded as 15.5% nucleotide sequence variation between the two species.

One way of assessing the significance of this figure of about 15% is to compare it with the extent of divergence of the respective coding regions. There is, however, insufficient sequence data available at this time to make such a direct comparison possible. Alternatively the coding regions of these mRNAs may be considered in two parts: those nucleotides that on mutation would cause an amino acid replacement, and those that would not (neutral mutations). From amino acid sequence data on rabbit and human globins²¹ the number of mutations causing amino acid substitutions between the two species may be calculated as 5.9%. Furthermore, Paddock *et al.*²² have compared their limited sequence data on rabbit globin mRNA with the sequences determined for human globin mRNA by Marotta *et al.*²³; and have suggested that the degree of neutral mutation is much larger, as much as 55%. Further sequence data are required to check this figure. From this preliminary conclusion it follows that the 15% divergence shown here in the 3' non-coding regions is a larger divergence than is allowed in those nucleotides which would change the amino acid sequence, but a smaller divergence than occurs in the degenerate (neutral) nucleotides. The significance of this in terms of function is probably that the 3' non-coding region is under less selective pressure than the nucleotides specifying amino acid sequence. This is perhaps not surprising. But equally the 3' non-coding region is much more conserved than are the nucleotides which are degenerate in the triplet code.

The above conclusions are preliminary because the complete 3' non-coding sequence is unknown. Indeed it is quite

Fig. 4 Comparison of human and rabbit globin mRNA sequences: *a*, α mRNA; *b*, β mRNA. H, Human; R, rabbit; X, base change between human and rabbit; +, addition of sequence; —, deletion of sequence. With α -globin mRNA sequences, termination regions for the two species are also compared and the position of the carboxy-terminal amino acid sequence of α globin (the same for both species, see ref. 23) is positioned. The mRNA sequence connecting the termination regions with the 3'-terminal regions is indicated by a line. The nucleotide sequence determination of the rabbit and human globin mRNA 3'-terminal regions is described in detail elsewhere (ref. 7 and N.J.P. and J. I. Langley, unpublished). The termination sequence for the rabbit α -globin mRNA has been determined by N.J.P. (unpublished), whereas the human α -globin termination sequence has been established by others^{19,21}.



possible that only the termination region and the sequence adjacent to poly(A) is conserved at the 15% level, whereas there is a greater sequence variation in the rest of the 3' non-coding region.

Discussion and conclusions

The A-A-U-A-A-A sequence present in all six mRNA 3' terminal sequences seems to display the most significant sequence homology between mRNAs. As this sequence has been demonstrated in four different species—human, mouse, rabbit and chicken—it seems likely that it is present in all eukaryotic mRNA, although further work will be necessary to be completely certain. The biological functions of this sequence remain unknown, although one may speculate from its proximity to the 3' end of the mRNA that it is a signal for an enzyme involved either in the synthesis or processing of mRNA from its presumed precursor⁴. The conservation of sequence between the 3' non-coding sequences of human and rabbit globin mRNA further suggests that much of this non-coding sequence, in addition to the A-A-U-A-A-A sequence, might possess defined sequence-specific functions. As much of the non-coding sequences described above are not homologous for different mRNAs, however, these functions may be specific to individual mRNAs rather than common to all.

The direct sequence analysis of complementary DNA (cDNA) has provided, and no doubt will continue to provide, a powerful method for the sequence determination of eukaryotic mRNA. This method, as with others^{23,24}, has, however, certain limitations. In particular the 5'-terminal regions of mRNA are particularly inaccessible when priming the synthesis of cDNA with oligo(dT) hybridised to the 3' terminal poly(A). It may be feasible, however, to over-

come this problem by using primers complementary to other regions of mRNA. Also, it is now possible to sequence cDNA that has been inserted into a bacterial plasmid²⁵⁻²⁸. Such DNA sequences are in a double-stranded state and are therefore susceptible to restriction enzyme digestion and the new rapid DNA-sequencing procedure described by Sanger and Coulson¹⁹.

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letters to nature

Effects of physical adsorption on porous interstellar grains

SOLID grains in space are likely to be highly porous in character. Data from meteorites¹, micro-meteoritic trajectories², as well as from comets³ provide indirect evidence that Solar-System grains are porous and a similar porosity is to be expected for interstellar grains. This would have important consequences for interstellar molecular hydrogen formation, and for the expected densities of noble gases in meteorites.

The effects of porosity in laboratory materials such as zeolites, silica gels and active carbons have been studied for many years⁴, mainly with a view to exploiting their properties as adsorbents and molecular sieves. Adsorbent pores are voids in solids which usually communicate with each other. Pore sizes range from 1,000 Å (macropores) to a few Å (micropores). The distribution of pore sizes to be expected in astrophysical solids depends on the mechanism by which they form. The sticking together of separate refractory grains embedded in a matrix of volatile ices or polymers which subsequently partially evaporate because of heating, could result in macroporous structures, as shown in micro meteorites (mass densities $\sim 0.3 \text{ g cm}^{-3}$). Microporosity with pore dimensions $< 10 \text{ Å}$ may result from the thermal dehydration of originally hydrated mineral crystals (for example zeolites or silica gels).

For macroporous grains the total effective adsorption area could be increased by an order of magnitude, but the physisorp-

tion energy remains unaltered. Microporosity, on the other hand, leads to enhancement of effective physisorption energy by a factor of ~ 5 in a typical case (Fig. 1, ref. 4), together with an increase of the adsorption area of a grain by a few orders of magnitude. The surface area per unit mass for non-porous spherical grains of radius a , density s is $3/as$. For typical values $a = 1.5 \times 10^{-6} \text{ cm}$, $s = 2 \text{ g cm}^{-3}$, this gives a 'specific adsorption area' $10^6 \text{ cm}^2 \text{ g}^{-1}$. In the case of a microporous material (for example zeolites) an average value for the specific adsorption area is $1.5 \times 10^7 \text{ cm}^2 \text{ g}^{-1}$, a factor 150 greater than for non-porous grains. This enhancement is interpreted as resulting from a volume-filling effect. The consequences of these properties for the formation of hydrogen molecules in interstellar space and for the retentive siting of noble gases in meteorites are examined below.

The condition for efficient H_2 formation on a grain surface is that the residence time of an adsorbed atom exceeds the mean time interval between successive arrivals of H atoms. This gives a maximum grain temperature

$$T_m = -\frac{q}{\ln(\alpha \langle v \rangle n_H \sigma \tau_0)}$$

where q is the binding energy (Kelvin) of an H atom, α is the sticking coefficient, $\langle v \rangle$ is the mean H atom velocity, σ is the grain cross-sectional area, and τ_0 is a characteristic desorption lifetime. Early estimates⁵ including the effect of zero-point

energy in the adsorption energy, with $q = 200$ K, yielded $T_m \approx 6$ K for typical interstellar conditions. This value of grain temperature is unacceptably low. Even the later calculations of Hollenbach and Salpeter⁶ which give $q \sim 400$ K (for hydrogen adsorbed on water ice) and $T_m \sim 12$ K allow molecular formation to occur only in clouds where dust shielding is significant. To increase T_m they proposed sites of greater binding on the grain, because of the presence of impurities and dislocations, with $q \sim 2,000$ K. For the limited number of these sites they chose, T_m increased to 40 K. Microporosity can, however, lead to an increase in q at all sites in all micropores. An increase by a factor of ~ 5 gives maximum grain temperatures of ~ 30 K. Even higher values of T_m are likely since the value $\tau_0 = 6 \times 10^{-13}$ s used in earlier calculations is based on the Frenkel theory of adsorption for non-porous surfaces. An increase of τ_0 by several orders of magnitude, likely for porous materials, would lead to $T_m \gg 30$ K. No dust shielding is then required, and H_2 recombination could take place on relatively 'hot' grains, even in intercloud regions. Such an effect could be important in accounting for recent Copernicus observations⁷.

Porous grains will have a major role in the origin of primordial noble gases in meteorites. An outstanding problem concerning the origin of noble gases in meteorites is the enormously large nebular pressures needed to account for the observed abundances in carbonaceous chondrites, if one assumes equilibrium solubility models^{8,9}. Using the experimental data of solubilities in magnetite, the pressures needed to obtain the observed amounts of rare gases are 5–8 orders of magnitude larger¹⁰ than might be reasonably estimated¹¹. To overcome this difficulty it has been hypothesised⁸ that the equilibrium solubility of noble gases are 5–8 orders of magnitude larger in the minerals where these gases are found than in magnetite. Alternatively, it has been proposed that the noble gases may be trapped by physical adsorption, the need for a hypothetical increase in solubility thus being avoided. The nebular pressures required for such adsorption are marginally plausible¹² within the framework of detailed numerical models for the condensation of the solar nebula¹¹. Furthermore, the observed elemental pattern of the noble gases is well reproduced by an adsorption process.

This model, however, based on measurements made on the Allende meteorite, suffers two major drawbacks¹⁰. Extensive heating¹³ and etching¹⁴ experiments on several meteorites indicate that the associated gases are firmly bound, and seem to be uniformly distributed throughout the host crystal. These facts may be taken to imply that the role of adsorption in the conventional sense is minimal.

The presence of micropores will alter this picture. The retentivity will increase enormously from an increase in the physisorption energy. Further, the micropores will allow the gases to fill the entire volume of grains as opposed to some specific surfaces alone. Of course, the evidence gathered so far from meteorites indicates merely the existence of pores. The spectrum of the radii of the pores has not yet been determined. The above physical arguments strongly suggest, however, that the minerals containing the noble gases will be highly microporous. An experimental determination of the degree of microporosity of chondrites is thus timely and highly desirable.

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Photon correlation study of stellar scintillation

DURING the past decade photomultiplier-tube technology and signal processing using integrated circuits have progressed to the point at which the analysis of fluctuating light signals can now be carried out digitally, in real time, with accuracy close to the theoretical limit. These 'photon correlation' techniques have so far found their main application in laser light-scattering spectroscopy¹ and laser doppler velocimetry². The techniques need not, however, be limited to applications involving lasers and can be used for the analysis of fluctuating optical signals of any origin (provided the fluctuation time is $\geq 10^{-8}$ s). Here we report a preliminary study of the statistical and temporal correlation properties of stellar scintillation, taking advantage of the fast response time and high sensitivity of equipment now standard for laser light-scattering applications.

The twinkling of stars is, of course, a phenomenon of long standing interest and a continuing source of controversy. It is caused by the presence of fluctuations in the refractive index of the Earth's atmosphere, but the exact nature and role of these fluctuations seems to be in some dispute^{3,4}. The twinkling phenomenon is associated with a fluctuation in amplitude, or scintillation, of the optical intensity incident on the eye. This is a potential source of information and can be used as a remote probe of the atmosphere just as radio-star scintillation has been used to investigate the ionosphere⁵. There is indeed a good deal of current interest in this aspect of the phenomenon^{6,7}. Unfortunately, it is also a form of noise which may limit the resolution of astronomical telescopes. The current development of stellar speckle interferometry⁸ as a means of overcoming conditions of bad seeing therefore provides further motivation for a quantitative characterisation and understanding of stellar scintillation.

Observations were made in late January, 1976, at RSRE, Malvern (52°8'N, 2°20'W). The results on Sirius (α Canis Majoris) reported here were obtained between 2030 and 2200

Fig. 1 Normalised intensity correlation function of scintillations in light received from Sirius, combining the results of several measurements taken over a 1.5-h period, not corrected for background. *a*, 5-nm filter; *b*, without filter.

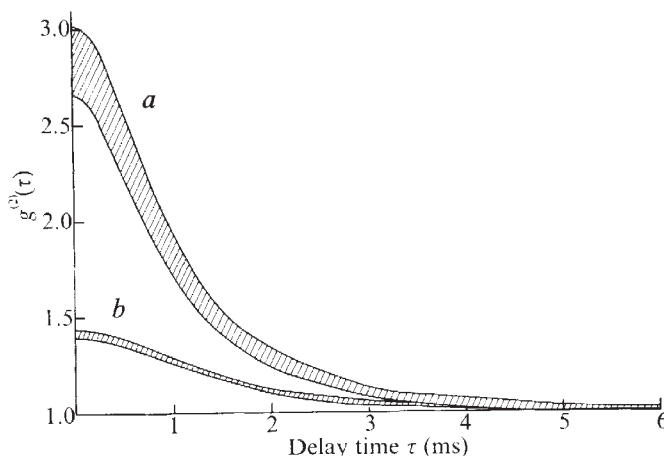


Table 1 Normalised factorial moments $n^{[m]}$ of photon-counting distribution, that is, normalised moments of the intensity fluctuation distribution¹

m	Uncorrected*	$n^{[m]}$ Corrected†	Gaussian‡
2	2.74 ± 0.13	2.89 ± 0.14	2
3	16.9 ± 2.2	18.8 ± 2.5	6
4	191 ± 47	223 ± 55	24
5	$3,226 \pm 1,220$	$3,942 \pm 1,490$	120

*Moments of measured distribution.

†Moments corrected for presence of background light ($\sim 4\%$ total intensity) assumed to be non-fluctuating on time scale of interest.‡For a Gaussian field, $n^{[m]} = m!$ (ref. 1).

on January 20 with the star coordinates near 165° azimuth and 21° elevation. Qualitatively similar results were obtained with Vega (α Lyr) during the same period. A blustery westerly airstream covered the region at this time with wind speeds of the order of 10–15 knots near the ground. An ITT FW130 (S20) photomultiplier, preceded by an 18.5-cm focal length telephoto lens and a filter centred at 443 nm with bandwidth 5 nm and 20% transmission, was mounted about 40 feet above the ground on a radar dish programmed to track the star. The star was imaged with $f/22$ optics (0.84-cm diameter aperture) on to a field stop of diameter 1 mm; this was followed by a dispersing lens which distributed the received light over the photocathode of the detector. The photomultiplier output was thus a measure of the light intensity falling on an area of about 0.55 cm^2 . Photon detection rates were typically greater than $2.5 \times 10^4 \text{ s}^{-1}$ when the star was in the field of view and of the order of 10^3 s^{-1} when it was not, made up of 10^2 s^{-1} room temperature dark count and $9 \times 10^2 \text{ s}^{-1}$ sky background. The optical assembly, including telephoto lens, viewing system, phototube and amplifier-discriminator circuitry, and the digital correlator used to measure photon statistics and correlations, were standard commercial items (Malvern Instruments, UK). For correlation measurements the correlator was operated in the single-scaled mode⁹, which provided a direct measurement of the normalised intensity correlation function, $g^{(2)}(\tau) = \langle I(0)I(\tau) \rangle / \langle I \rangle^2$ where I is the light intensity and τ the delay time, whatever the statistics of I .

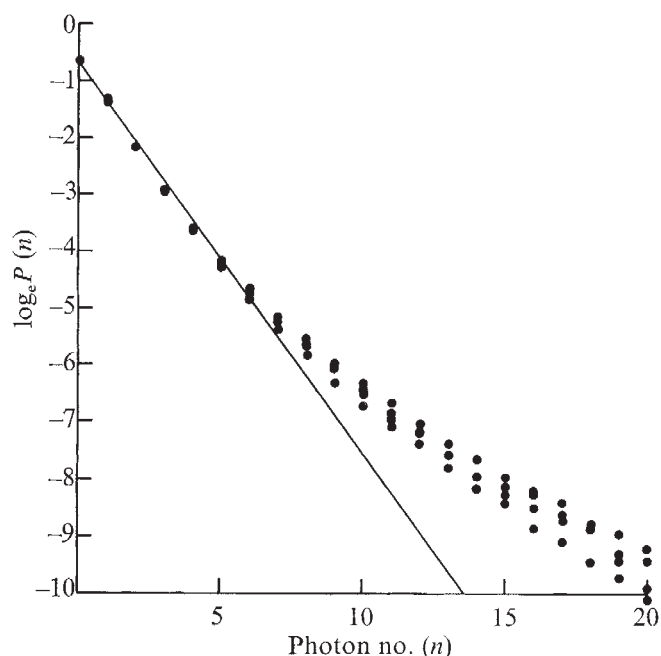
Fig. 2 Probability distribution of photon detections. Experimental points would lie on the straight line if the received light field were Gaussian distributed with the same mean of about 1 count per 40- μs sample time.

Figure 1a shows a composite correlation function obtained from several measurements of 1–2-min duration with sampling times varying from 20 to 200 μs . A band roughly 2 s.d. wide indicates run-to-run variations which arise from both finite counting times and non-stationary atmospheric conditions. The normalised contrast

$$\lim_{\tau \rightarrow 0} g^{(2)}(\tau) - 1$$

corrected for background, is about 1.9, significantly greater than the value of unity expected for Gaussian light. The non-Gaussian nature of the fluctuations is also reflected in the higher-order intensity moments (Table 1) and the photon-counting probability distribution (Fig. 2). A considerably reduced contrast of ~ 0.42 was observed, however, when the colour filter was removed (Fig. 1b). The width at half-height of the correlation function is about 10^{-3} s implying a characteristic fluctuation time much shorter than the commonly accepted value for the response time of the eye. Taking this to be of order $1/25 \text{ s}$, however, a r.m.s. contrast of about 10% after temporal integration of the whole visible spectrum is indicated or 20% if integration is restricted to a 5-nm band. Since the eye perceives fluctuations in colour, the latter figure would seem to be appropriate for comparison and not inconsistent with visual observations, although physiological effects due to image motion on the retina cannot be ruled out¹⁰.

To our knowledge non-Gaussian fluctuations giving contrast in excess of unity have not been observed in any previous measurements of stellar scintillation¹¹, probably because in earlier experiments averaging of the intensity pattern occurred through the use of large apertures, insufficiently narrow filters or simply slow response time instrumentation. High contrast intensity patterns are known to be produced in certain conditions when an infinite plane wave is scattered by a random phase screen or inhomogeneous medium¹². In particular, a random-phase screen characterised by a single length scale and introducing path differences greater than about one-third of a wavelength produces large intensity fluctuations in the region of focusing determined by the lens-like behaviour of individual refractive index inhomogeneities¹³. Our results seem to be consistent with recent theoretical predictions based on this interpretation of the origin of stellar scintillation¹⁴. The phenomenon of focusing, however, is highly model dependent^{12,15} and further experiments are essential if the role of the various turbulent layer structures present in the atmosphere is to be fully elucidated.

Finally, it is worth noting that the shadow patterns observed in the receiving plane of large aperture telescopes¹⁶ are, in conditions of high contrast, probably smeared-out caustics. The presence of many of these within the aperture would indicate that light from many (strong) atmospheric scatterers was entering the optical system leading to near-Gaussian speckle in the image⁸.

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Q in cosmology

THE following example in classical mechanics yields the key equations of relativistic cosmology, including the new equations by Harrison¹. The argument given here develops that of an earlier note².

A set of n gravitating particles of masses m_i are at positions $\mathbf{r}_i(0) = \mathbf{a}_i$ relative to a fixed origin at the time $t = 0$, when an explosion gives a radial motion $\mathbf{r}_i(t) = R(t)\mathbf{a}_i$ to all the particles. The scale factor R is the same for all the particles. The kinetic energy of the system at time t is a sum of terms of the type $\frac{1}{2}m_i v_i^2$, and the gravitational potential energy is a sum of terms of the form $(|\mathbf{a}_i - \mathbf{a}_j| = a_{ij})$

$$-G \frac{m_i m_j}{r_{ij}(t)} = -G \frac{m_i m_j}{a_{ij}} \frac{1}{R(t)}$$

Accordingly, the energy equation of the system is

$$E = A\dot{R}^2 - B/R, \quad A \equiv \frac{1}{2} \sum_i m_i a_i^2, \quad B \equiv \frac{G}{2} \sum_{i \neq j} \frac{m_i m_j}{a_{ij}} \quad (1)$$

To attain a formal analogy with relativistic cosmology, write

$$E/A \equiv -kc^2, \quad B/A \equiv C,$$

and add a cosmological energy term $A\lambda R^2/3$. This gives the following energy equation and its first and second time derivatives

$$\frac{\lambda}{3} = H^2 + \frac{kc^2}{R^2} - \frac{C}{R^3} \quad (H \equiv \dot{R}/R) \quad (2)$$

$$\frac{\lambda}{3} = \frac{C}{2R^3} - qH^2 \quad (q \equiv -\ddot{R}R/\dot{R}^2) \quad (3)$$

$$\frac{\lambda}{3} = QH^2 - \frac{C}{R^3} \quad (Q \equiv R^2 \ddot{R}/\dot{R}^3) \quad (4)$$

The importance of the Q parameter has already been noted¹. One obtains the basic cosmological differential equation in two forms which are independent of the experimentally little known parameter λ , by equating equations (2) and (3) or by equating (2) and (4)

$$\frac{kc^2}{R^2} = \frac{3C}{2R^3} - (q+1)H^2 \quad (5)$$

$$\frac{kc^2}{R^2} = (Q-1)H^2 \quad (6)$$

Adding twice (3) to (4) and taking (3) from (4):

$$\lambda = (Q-2q)H^2, \quad \frac{3C}{2R} = (Q+q)H^2 \quad (7,8)$$

In a Newtonian context, the system is ever expanding if E is positive; that is, if k is negative. By equation (6), the appropriate condition is $Q < 1$, and for $\lambda = 0$ models, it is equivalent to $q < \frac{1}{2}$ (equation (7)). The inequalities are reversed if the system is to collapse again. Note that only if one puts (ρ is the mass density)

$$C = \frac{8\pi}{3} G\rho R^3 \quad (9)$$

do these become the equations given by Harrison¹. The simplicity of the example given here is illuminating: the role of q , which determines whether a ($\lambda = 0$)-model is closed or open, can clearly be taken over by Q if $\lambda \neq 0$. A continuous distribution of gravitating matter, which is needed to obtain equation (9) in general relativity, and which was used in ref. 1, is clearly not needed here. A discrete distribution of galaxies with sufficient symmetry to ensure the continued validity of Hubble's Law is all that is required. It is to be emphasised that in Newtonian cosmology it is possible to give discussions of interacting particles and discrete matter distributions which give Hubble's Law, though the corresponding general relativistic problems remain unsolved.

This possibility has been noted already^{2,3}, but no example has been given before. The simplest distribution to verify this possibility is a set of eight galaxies, each of mass m , on the vertices of a cube of side $2a_0$, which are given velocities, v , radially away from the centre of the cube. Because of the seven other galaxies each galaxy is then subject to a deceleration in the direction of the centre. Its magnitude is

$$\frac{D}{2a(t)^2}, \quad D \equiv \left[1 + \frac{\sqrt{2}}{2} + \frac{\sqrt{3}}{9} \right] \frac{Gm}{2}$$

where $2a(t)$ is the side of the cube at time t . If the escape velocity $v_{\text{esc}} = \sqrt{(D/a_0)}$ has been exceeded, the Hubble parameter is

$$\frac{\theta^3(1+w^2)^{\frac{1}{2}}}{Dw^3}, \quad \theta \equiv (v^2 - v_{\text{esc}}^2)^{\frac{1}{2}}$$

$$w \equiv \theta\sqrt{[a(t)/D]}.$$

Essentially, a topic for so far undiscovered theorems in Newtonian physics is raised here: the classification of (usually unstable) particle configurations which, for a given interaction, are consistent with a Hubble type expansion; and expressions for the Hubble parameter should also be found in each case.

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Anomalous M₁ tide at Lagos

I HAVE recently described¹ the phenomenal appearance in the seas off western Europe of a tidal component with a period of exactly one mean lunar day, namely the "M₁ tide". Although only a few mm in amplitude, it is about ten times larger than one would expect from comparison with ordinary diurnal tides, and suggests a resonant response of

part of the Atlantic Ocean to forcing functions which are symmetrical with respect to the Equator. The forcing function for the ordinary diurnal tides is antisymmetric, and its components are relatively suppressed, enhancing the appearance of M_1 in this sea area.

Most of the places for which I showed evidence of an enhanced M_1 tide, however, were in the shelf seas surrounding the British Isles. The one notable exception was Terceira (Azores), where the results were somewhat marginal. It might therefore have been suggested that the phenomenon is not really oceanic but is confined to the north-west European shelf, although I gave some reasons why this seemed unlikely. I now describe results which confirm the oceanic scale of this effect.

Resolution of the true M_1 tide requires at least 5 yr, and preferably 9 yr of data. A tidal record of more than 9 yr duration has been obtained from Lagos on the south Portuguese coast (near Cape St Vincent; $37^{\circ}06'N$, $8^{\circ}40'W$), through the cooperation of the Instituto Geografico e Cadastral at Lisbon. The tide gauge, being maintained for geodetic purposes, is excellent in quality, and analysis of the records shows them to have very low noise level and a virtual absence of nonlinear effects. The true M_1 tide stands out clearly from its spectral background, with an amplitude of 5 mm and Greenwich phase lag G of 245° , representing an admittance to the normalised generating function² of 1.23. By contrast, the major component of the ordinary diurnal tide, at a frequency close to 1 cycle per lunar day but actually $1/8.85$ cycles yr^{-1} greater than that of M_1 , has amplitude 4 mm, $G=5^{\circ}$, representing an admittance to its generating function of only 0.19. Lagos is far enough from Britain not to be affected by resonances in British shelf seas, should they exist, so a significant part of the north-eastern Atlantic must be involved. Also, a definite northwards progression of phases across the eastern oceanic shelf edge now emerges, as seen by the following list of phase lags G for the M_1 tide: Lagos, 245° ; Brest, 268° ; Scilly, 276° ; Malin, 312° ; Stornoway, 308° ; Lerwick, 330° .

Apart from a slight anomaly at Stornoway, the trend of the phases is in keeping with the progression of the normal mode of the Atlantic/Indian ocean system at 23.5-h period, computed by Platzman³, which I cited earlier¹ as an explanation of the M_1 phenomenon. Platzman's mode progresses in phase by 45° roughly between the positions of Lisbon and the Faeroes. G for Terceira is 275° , about the same as Scilly, indicating a cotidal line stretching south-west from Scilly. This, together with the reduced amplitude at Terceira, again supports Platzman's modal diagram.

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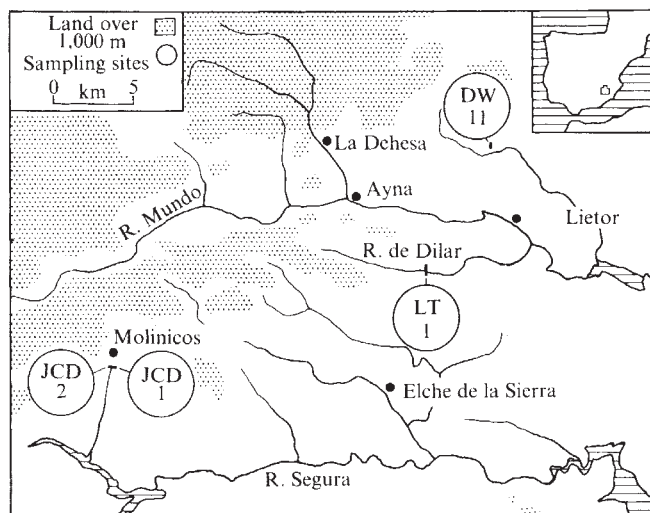


Fig. 1 Location of Spanish sampling sites discussed in text.

deposition throughout the Mediterranean Basin and Near East.

The Mundo is a tributary of the Segura², which drains into the Mediterranean south of Alicante. Three Quaternary valley deposits can be identified in the reaches shown in Fig. 1. The oldest, for which I propose the name Ayna Tufa, has a maximum thickness of 17 m above Ayna, where it overlies Mesozoic limestone and grades upstream into a well stratified marl. The tufas and marls are surmounted by a deposit which takes the form of cemented scree at Ayna and of a valley fill below Ayna and in many other valleys in the area. This unit, for which the name Elche Formation is proposed, attains its maximum observed thickness of 11 m in the Rambla de Dilar; it is generally characterised by subangular gravel in a red, sandy matrix and includes horizons strongly cemented by calcium carbonate. Channels cut into the Elche sediments have been partially filled, to a maximum observed depth of 2.5 m, by a well bedded deposit composed of silts, sands and gravels for which the name Liétor Alluvium is proposed. This unit is at present undergoing trenching.

A more complex Quaternary succession has been reported from the central and southern parts of the Province of Alicante³ but secure ¹⁴C dates are still not available for it. Elsewhere in south-eastern Spain the dating of continental deposits is based largely on their relationship to fossil beaches whose age is in dispute or on correlation with other areas^{4,5}. Four ¹⁴C dates were obtained during the present study (Table 1). The first was on charcoal from a slightly disturbed hearth near the base of the Elche Formation south of Molinicos (JCD 2). It is worth adding that at Ayna the cemented scree yielded a single Palaeolithic artefact, and that concentrations of Mesolithic material were found on the surface of the Elche Formation at various localities near La Dehesa. The Liétor Alluvium supplied datable charcoal in a gravel pit near Liétor (DW 11), in the Rambla de Dilar, where it forms a terrace remnant (LT 1), and south of Molinicos, where it is represented by colluvium overlying the Elche Formation (JCD 1). The three dates tally satisfactorily, and indicate an age of about 700 yr for the mid-point in aggradation.

A threefold post-Tertiary valley sequence similar to that outlined here has been reported from numerous valleys in other parts of the Mediterranean Basin and in the Near East^{6,7} and also in central Périgord⁸. It has been suggested⁶ that the tufa reflects greater spring discharges than those of today, that the older of the alluvial fills points to active frost weathering in the uplands coupled with the incidence of intense but short-lived and infrequent rains comparable to those now prevailing at lower latitudes, and that accumulation of the younger fill was in response to a temporary southward shift in the depression belts of Europe. The onset of the last of the three episodes

Diachronism in Old World alluvial sequences

THE diachronism of an environmental change—that is, the extent to which its age varies with location—is a useful clue to its origin¹. In the case of alluvial sequences, the time taken for filling and trenching to work their way through a drainage basin is likely to obscure chronological differences between basins unless they are sufficiently distant from each other for any regional trend to become apparent. I report here ¹⁴C dates for alluvial units in south-eastern Spain, and suggest that they are consistent with the effects of latitudinally diachronous

Table 1 ^{14}C dating of rock units

Rock unit	Sample no.	Laboratory no.	Grid reference	Location	Age (yr b.p.)	^{13}C : ^{12}C rel. PDB (‰)	Material	Remarks
Elche Formation	JCD 2	SRR-730	7274 × 4317	2°14'22"W 38°27'30"N	39,735 ± 1,190 —1,035	—22.2	Charcoal	At depth of 9 m in 10-m section
Lietor Alluvium	LT 1	SRR-726	7453 × 4363	20°0'46"W	819 ± 35	—23.2	Charcoal	At depth of 1.5 m in 2.5-m section
		SRR-727		38°30'39"N	853 ± 50	—23.4	Charcoal	Repeat analysis
Lietor Alluvium	DW 11	SRR-728	7492 × 4439	1°58'12"W 38°34'43"N	780 ± 110	—24.7	Charcoal	At depth of 1.5 m in 2.5-m section
Lietor Alluvium	JCD 1	SRR-729	7274 × 4318	2°14'22"W 38°27'30"N	419 ± 65	—25.0	Charcoal	At depth of 1 m in 3-m section

varies by up to 500 yr according to location. This has been cited in support of the view that aggradation stemmed from man-induced soil erosion⁹, but a preliminary plot against latitude of the ^{14}C dates then available for the deposit indicated a latitudinal decrease in its age which seemed to favour the 'cyclonic' explanation¹⁰.

Figure 2 extends this approach to the other two units. It incorporates the Spanish material and all the other ^{14}C dates whose attribution is certain, as well as limiting archaeological ages derived from historical sites or from excavated material locally calibrated with the help of radiocarbon dating. Granted that the number of points remains small, that they include some (notably those on tufa) which are open to challenge, and that no correction has been made for altitude or other possible

distorting factors, there is some indication of a northward lag in the transition from tufaceous to alluvial deposition, and of a southward lag in the onset of the second alluvial phase. The graphs also suggest that in both instances aggradation terminated more abruptly than it began, just as the trenching of individual valley fills often progresses more rapidly than does their accumulation¹¹. Figure 2 could be taken to imply that the climatic conditions responsible for the older of the alluvial fills emanated from lower latitudes, whereas those that produced the younger fills stemmed from higher latitudes, both inferences being in accord with the interpretations prompted by the field evidence. It remains to be seen whether additional ^{14}C dates will support the graphs so that their gradients can be used to test palaeo-climatic hypotheses with some confidence.

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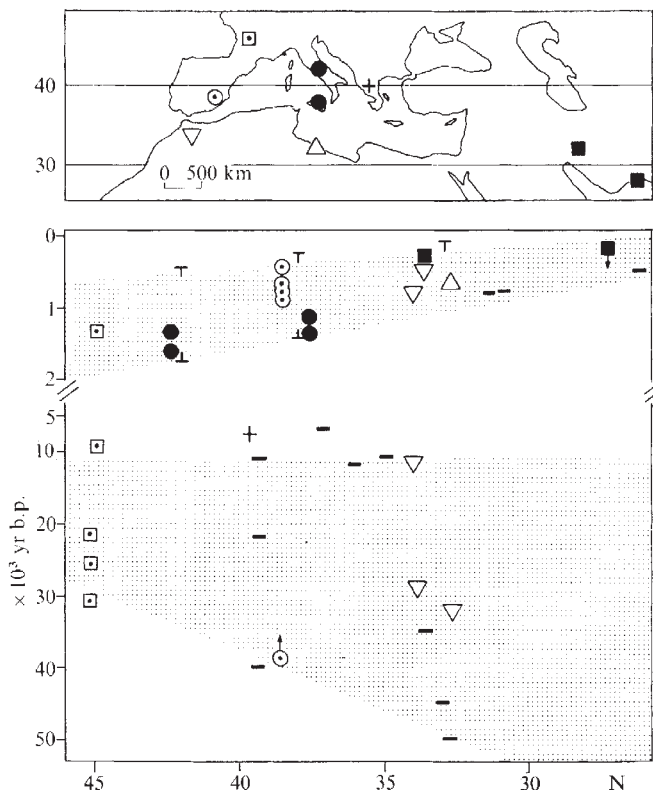


Fig. 2 Plot of age against latitude and provenance of ^{14}C dates for the three deposits reviewed. The upper stippled zone includes the dates for the younger alluvial fill, the lower stippled zone those for the older fill, the blank area between them those for the intervening erosional phase, and the blank triangle at the base those for the tufa. The only dates which do not conform to this scheme are the two from the Dordogne (◻) within the lower stippled zone, as they are on tufa, but this tallies with field evidence for overlap between tufaceous and alluvial deposition in that area⁸. Archaeological ages (—) include some that immediately predate (Δ) or postdate (◻) the younger fill. Data from this article and from refs 6–9 and 12.

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Effect of mineral precipitation on isotopic composition and ^{14}C dating of groundwater

I PRESENT here a method for determining the combined effects of concurrent mineral precipitation and dissolution on the carbon isotope composition of groundwater. The results are applied to the 'adjustment problem' in the ^{14}C dating of groundwater, and are illustrated using published data from the London Basin chalk aquifer. Some ^{14}C ages are changed by more than 10,000 yr.

'Raw' ^{14}C data from groundwaters must be adjusted to allow for dilution attributable to the dissolution of bedrock

carbonate which contains no ^{14}C . Either stable isotope measurements or conventional geochemical measurements may be used to determine the adjustment factor. In both cases 'initial' (that is, at the start of conditions which are closed to carbon dioxide) values of either ^{13}C content ($\delta^{13}\text{C}$) or total dissolved inorganic carbon molality ($m\text{C}$) must be known¹. These initial values are often difficult to estimate. In addition, even adjusted ages are subject to errors arising from various forms of contamination within the aquifer². Nevertheless, provided the aquifer geochemistry is well understood, reasonably reliable ages can be determined.

One important and relatively common process has either not been considered, or been considered inadequately, in past studies of carbon isotopes in groundwater: the concurrent dissolution and precipitation of two minerals (that is, incongruent dissolution, if both are carbonate minerals). Because of isotopic fractionation between aqueous phase and solid carbon-containing species, the isotopic composition of any precipitate will differ from that of the parent solution. For dissolution, the dissolved material will generally differ isotopically from the solute. Thus, both precipitation and dissolution must, in general, alter both the stable and unstable isotope composition of a groundwater. This paper presents the first quantitative estimate of the combined effects of these processes.

Let $m\text{D}$ be the contribution to changes in $m\text{C}$ over a flow-path attributable to dissolution of one mineral, and let $m\text{P}$ be the contribution attributable to precipitation of another mineral. Over a flow-path increment the change in mass of ^{13}C carried by the groundwater must balance the net gain attributable to dissolution and loss attributable to precipitation. This mass balance may be expressed mathematically as

$$d(m\text{C}\delta^{13}\text{C}) \approx \delta^{13}\text{C}_s d(m\text{D}) - \delta^{13}\text{C}_p d(m\text{P})$$

where 's' and 'p' denote bedrock and precipitate values. In terms of the fractionation factor between precipitate and solution

$$F = \delta^{13}\text{C}_p - \delta^{13}\text{C}$$

this becomes

$$m\text{C}d(\delta^{13}\text{C}) + \delta^{13}\text{C}d(m\text{C}) \approx \delta^{13}\text{C}_s d(m\text{D}) - (\delta^{13}\text{C} + F)d(m\text{P})$$

If $\alpha = d(m\text{D})/d(m\text{P})$ is the relative dissolution to precipitation contribution to $m\text{C}$, then, for $\alpha = \text{constant}$ and $F = \text{constant}$, and noting that $d(m\text{C}) = d(m\text{D}) - d(m\text{P})$, the above can be integrated to give

$$\delta^{13}\text{C} - \delta^{13}\text{C}_s \approx X(\delta^{13}\text{C}_0 - \delta^{13}\text{C}_s) + F(X-1)/\alpha \quad (1)$$

where

$$X = \left\{ \frac{m\text{C}}{m\text{C}_0} \right\}^{\alpha/(1-\alpha)}, \quad \alpha \neq 1$$

$$X = e^{-m\text{P}/m\text{C}_0}, \quad \alpha = 1$$

here, '0' denotes the value at the commencement of concurrent dissolution and precipitation. This would normally be preceded by a dissolution-only period. In equation (1), $X(\delta^{13}\text{C}_0 - \delta^{13}\text{C}_s)$ is the contribution attributable to precipitation and dissolution ignoring fractionation, and $F(X-1)/\alpha$ is the fractionation effect. As α tends to infinity (that is, dissolution dominates) this term becomes small and the usual dissolution-only result obtains.

For ^{14}C , the corresponding relationship is

$$A \approx A_0 X \quad (2)$$

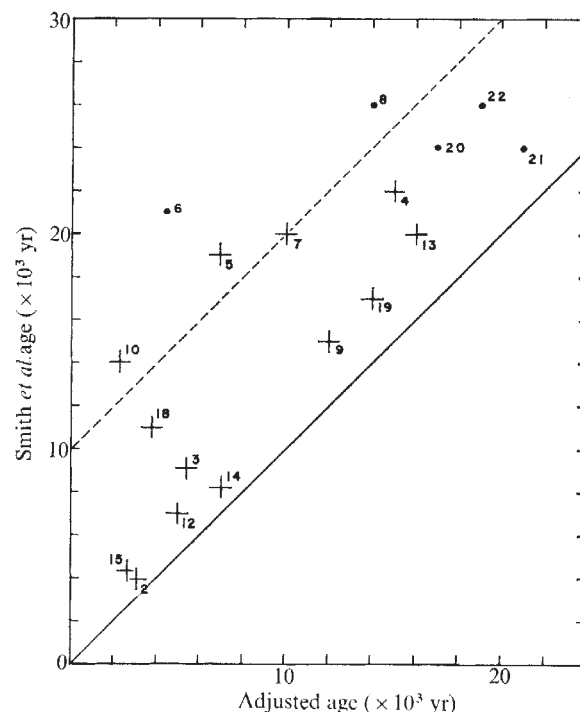


Fig. 1 Comparison of groundwater ages before and after adjustment for the effects of concurrent mineral precipitation and dissolution. Numbers, site numbers used by Smith *et al.*^{3,4}; solid circles, data values regarded by Smith *et al.* as having the greatest error margins. For points to the left of the dashed line the precipitation-dissolution adjustment is greater than 10,000 yr.

where A is the activity (in % modern). Detailed derivations of these results will be given elsewhere.

These results are based on the assumptions of constant α and F . Constant α implies constancy of the relative driving forces for dissolution and precipitation. Although made here in the interest of mathematical tractability, I believe that, in the absence of detailed geochemical information from particular aquifers, it is the best possible assumption. The fractionation factor, F , varies considerably with pH and temperature; however, at the relatively high pH values needed to allow concurrent dissolution and precipitation, F is largely independent of these variables and is approximately 2.5‰.

After extensive combined dissolution and precipitation (that is, X tending to zero), equations (1) and (2) show that the isotope concentrations tend to limiting values of $\delta^{13}\text{C}_s - (F/\alpha)$ and 0‰. Since $F \approx 2.5\text{‰}$ this allows $\delta^{13}\text{C}$ to approach the bedrock value closely. In addition, ^{14}C activity may become very low independently of groundwater age. As an example, if $\alpha = 1$, $\delta^{13}\text{C}_s = 0\text{‰}$, and $F = 2.5\text{‰}$, the limiting $\delta^{13}\text{C}$ is -2.5‰ , much higher than expected in the absence of mineral precipitation. This limit corresponds to a steady state where 0‰ carbon is added to the groundwater by dissolution, while 0‰ carbon (that is, the groundwater value plus 2.5‰ fractionation) is being lost at an equal rate by precipitation.

Equation (1) can be used to adjust ^{14}C ages and so give an idea of the magnitude of dissolution-precipitation effects on the ^{14}C dating of groundwaters. Ages adjusted in this way will, necessarily, differ from those obtained using the dissolution-only formula (that is, equation (1) with $F = 0$). The difference will be one half life when $X = F/[F - \alpha(\delta^{13}\text{C}_0 - \delta^{13}\text{C}_s)]$. Greater differences will occur for smaller values of X (that is, as $\delta^{13}\text{C}$ approaches its limiting value). Values of X less than the above correspond to $\delta^{13}\text{C}$ values within about 3‰ of the limiting value. Since age errors of one half life (or more) are quite significant, a proper consideration of precipitation effects can be extremely important. As $\delta^{13}\text{C}$ approaches its limiting value the adjustment factor becomes increasingly sensitive to small errors in the data and/or the model. Thus, unfortunately,

the uncertainty in the adjustment increases as the magnitude of the adjustment increases.

To illustrate the possible magnitude of this effect I have re-evaluated isotope data from the London Basin chalk aquifer given by Smith *et al.*^{3,4}. These authors state that the anomalously high values of $\delta^{13}\text{C}$ observed in this aquifer are most probably attributable to concurrent dissolution and precipitation. In adjusting ^{14}C ages, however, they did not take fractionation effects into account, and used the standard dissolution-only formula. In the London Basin, $\delta^{13}\text{C}_s \approx 2.35\%$. The chemical evolution of the groundwater seems to be closed-system dissolution (during which $\delta^{13}\text{C}$ evolves from a soil-zone value of -26% to around -13%) followed by concurrent dissolution and precipitation. The dissolution-only period reduces the ^{14}C activity to around 50%, so that the age may be estimated by

$$t = \frac{5,730}{\log 2} \log \left(\frac{50X}{A_m} \right)$$

where A_m is the measured groundwater activity and X is given (using equation (1) and assuming $\alpha = 1$) by

$$X = \frac{(-\delta^{13}\text{C} - 0.15)}{12.85}$$

Figure 1 compares the ages given by Smith *et al.*^{3,4} with ages recalculated using the relationship defined here. The differences are, in some instances very large: more than 10,000 yr in four cases. Adjustments of this magnitude are of considerable importance. In support of these calculations it is reassuring that none of the $\delta^{13}\text{C}$ values observed by Smith *et al.*^{3,4} exceeds the limiting value of -0.15% implied by equation (1).

Smith *et al.*³ have suggested that some of the London Basin water may be recharge dating from before the last glacial maximum. This suggestion can be re-examined in the light of the revised ages (Fig. 1). After the last glacial maximum, the earliest recharge to the aquifer probably occurred during the first post-glacial warm period around 13,000 yr b.p. Ages in excess of this would confirm the suggestion of Smith *et al.*^{3,4}. Although many ages are substantially younger after readjustment, some of the ages still, indeed, exceed 13,000 yr b.p.; however, in view of the large uncertainties involved in allowing for mineral-precipitation effects, and the proximity of many $\delta^{13}\text{C}$ values to the theoretical limiting value (when adjustment uncertainty is greatest), the possibility that all the waters are younger than 13,000 yr b.p. must remain open.

Nevertheless, a direct interpretation of the ages shown in Fig. 1 supports the suggestion of Smith *et al.*^{3,4}. Some of the readjusted groundwater dates do fall within the last glacial maximum period. Since recharge at this time was probably negligible, if these ages are correct then the relevant samples are most probably older waters 'contaminated' by mixing with post-glacial recharge.

In conclusion, mineral precipitation within an aquifer can have a very significant influence on the groundwater isotope geochemistry. Ignoring mineral precipitation, or neglecting isotopic fractionation during precipitation, may lead to estimates of ^{14}C ages which are many thousands of years too old. Stable carbon isotope data may be used to adjust ages, but the largest adjustments are those which are subject to the greatest uncertainty.

I thank R. A. Downing for providing a pre-publication copy of the London Basin results^{3,4}.

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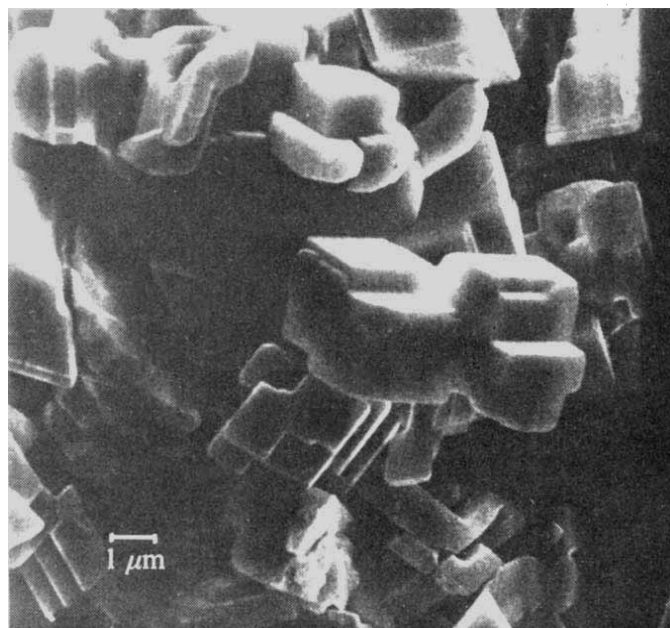
Calcium oxides of high reactivity

A RECENT study of the kinetics of decomposition of calcite (CaCO_3) single crystals *in vacuo* showed that if the reaction was interrupted before completion a 30- μm layer of a poorly crystalline material was present between the undecomposed CaCO_3 and a layer of normal polycrystalline CaO ¹. It was hypothesised that the material of this 30- μm layer is a metastable form of CaO that transforms irreversibly to the stable polycrystalline oxide when the accumulated strain exceeds a critical level. If so, the free energy of formation of the metastable oxide from the stable oxide must lie in the range between zero and $+31,500 - 21T \text{ J mol}^{-1}$ and might well lie at the positive end of this range². Such a metastable oxide should be chemically more reactive than the stable oxide. If the hypothesis is true, then the principal product of decomposition *in vacuo* of calcite particles of diameter $< 30 \mu\text{m}$ should be the metastable oxide. We present here evidence that this inference is correct, and we demonstrate that not only this oxide, but also a highly crystalline oxide which is produced when large calcite crystals are decomposed *in vacuo*, reacts more vigorously with water than does the product of calcite decomposition in air or nitrogen.

Calcite ground to a powder formed of block-like rather sharp-edged particles with cross sections ranging from 1 to 10 μm (Fig. 1) was decomposed in air or nitrogen in the range of temperatures between 650 °C and 800 °C. The product was composed of particles which had rounded surfaces and neck areas which indicate considerable sintering (Fig. 2). The product gives the diffraction pattern of highly crystalline CaO .

When the same CaCO_3 powder was decomposed *in vacuo* in the range of temperatures between 450 and 750 °C, there was little change in the apparent particle shape from those of the starting material, and little sintering (Fig. 3). It can be calculated from the relative molar volumes of CaCO_3 and CaO that the powder particles produced in vacuum decomposition, like the product of CaCO_3 single crystals *in vacuo*¹, must have $> 50\%$ porosity. Unlike the powders decomposed in air or nitrogen, the powders decomposed *in vacuo* were poorly crystalline, but did show weak diffraction peaks of the normal (NaCl type) CaO structure (Fig. 4). Heights of the (100) peak showed no

Fig. 1 Scanning electron micrograph of CaCO_3 powders.



¹ Wigley, T. M. L., *Wat. Resour. Res.*, **11**, 324-328 (1975).

² Pearson, F. J., Jr, and Hanshaw, B. B., in *Isotope Hydrology 1970*, 271-286 (International Atomic Energy Authority, Vienna, 1970).

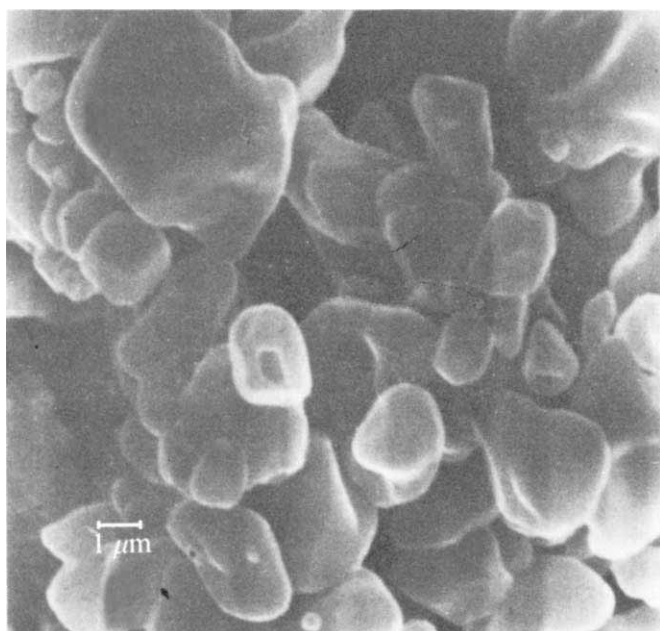


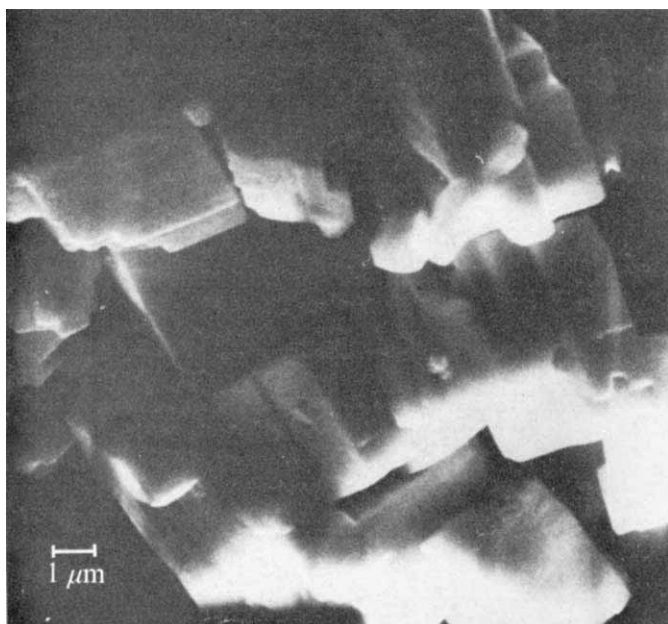
Fig. 2 Scanning electron micrograph of CaO produced from decomposition of CaCO_3 powders in dry nitrogen.

clear systematic dependence on time or temperature of heating (as high as $1,050^\circ\text{C}$).

The oxide produced by decomposition of CaCO_3 powder in air or nitrogen showed no measurable Ca(OH)_2 diffraction peaks when ground for 10 to 15 min in air and gained only 1% in weight when exposed to an atmosphere of water vapour at 140°C . In contrast, the poorly crystalline oxide produced by decomposition of the same powder *in vacuo* is completely converted to poorly crystalline Ca(OH)_2 by grinding in air for 10 min or by 3-min exposure to the water vapour stream at 140°C . The poorly crystalline hydroxide in turn transforms exothermally to the crystalline hydroxide when heated in air to $\sim 290^\circ\text{C}$.

Surprisingly, the product of complete decomposition of single crystals of calcite *in vacuo*, even though a CaO with a degree of crystallinity little different from that of the CaO produced by

Fig. 3 Scanning electron micrograph of CaO produced from decomposition of CaCO_3 powder *in vacuo*.



calcite decomposition in air, reacted with water vapour at rates that were not distinguishable by our hydration tests from rates for the poorly crystalline product of vacuum decomposition of CaCO_3 powders. This observation shows that the high rates of reactivity of the oxides prepared *in vacuo* are primarily consequences of the high internal surface areas of the block-like products of vacuum decomposition. The blocks produced *in vacuo* have $>50\%$ porosity¹, yet the pores are too small to observe at $\times 30,000$. Pore diameters probably average well under $0.01\ \mu\text{m}$ so that solid state diffusion over only short distances is necessary for the hydration reaction. The rounded forms of the oxide particles prepared by decomposition in air must be a consequence of extensive condensed phase diffusion, a process that would probably close the intra-particle pores.

In line with our original hypothesis that the $30\text{-}\mu\text{m}$ layer observed in the interrupted decomposition of a calcite single crystal *in vacuo* is a metastable form of CaO that transforms under sufficient strain to the normal crystalline form, the oxide

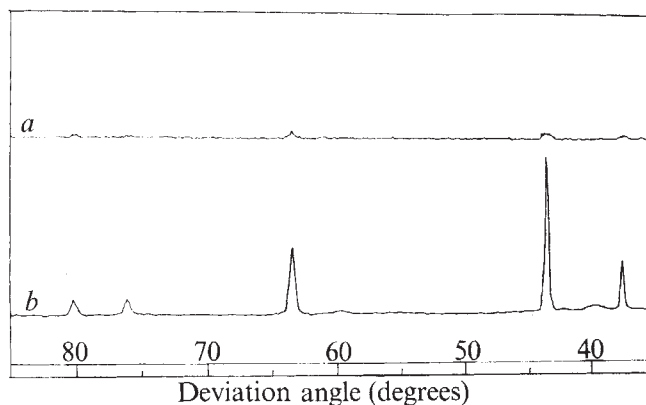


Fig. 4 X-ray diffractometer trace (cobalt $K\alpha$ radiation). a, CaO produced from decomposition of CaCO_3 *in vacuo*; b, CaO produced from decomposition of CaCO_3 powders in air.

of this layer is more reactive towards moisture than the other forms here identified. The X-ray peak which in the earlier study¹ was presumed to be a reflection for a metastable oxide is instead the strongest peak of the poorly crystalline hydroxide. The $30\text{-}\mu\text{m}$ oxide layer, after exposure to room temperature air for $<1\text{ h}$, shows only the diffraction peaks for the amorphous hydroxide. In the same conditions, the other products of *in vacuo* decomposition, if not ground, give patterns of either well crystallised or poorly crystalline CaO, as reported above.

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Wave power availability in the NE Atlantic

FOLLOWING Salter's¹ proposals for the extraction of energy from sea waves, we are working on the prediction of wave power output from devices situated at favourable coastal sites around the UK. While it is hoped that adequate spectral data from sites closer inshore will become available within the next year or so, the best present data for the North-east Atlantic are from O.W.S. India². We describe here the power available and, on simplified assumptions, the amount that might be extracted by ducks of various diameters. (The Salter 'duck' is a rocking cam-shaped device which is designed to give a high efficiency of energy extraction over a wide frequency band³.) Confirmation of the validity of the important assumption of additivity of power outputs from wave components of different periods is provided in a separate paper⁴.

The power, P , in a sinusoidal wave train in deep water is kTH_{rms}^2 per unit width of wave front, where H_{rms} is the root mean square displacement (that is, the standard deviation) of the water surface, T is the wave period and $k = \rho g^2/4\pi \approx 7.82 \text{ kW m}^{-3} \text{ s}^{-1}$. Thus for a mixed wave train of spectrum dS ($H_{rms}^2 = \int dS$), on linear theory

$$P = k \int T dS = k T_e H_{rms}^2$$

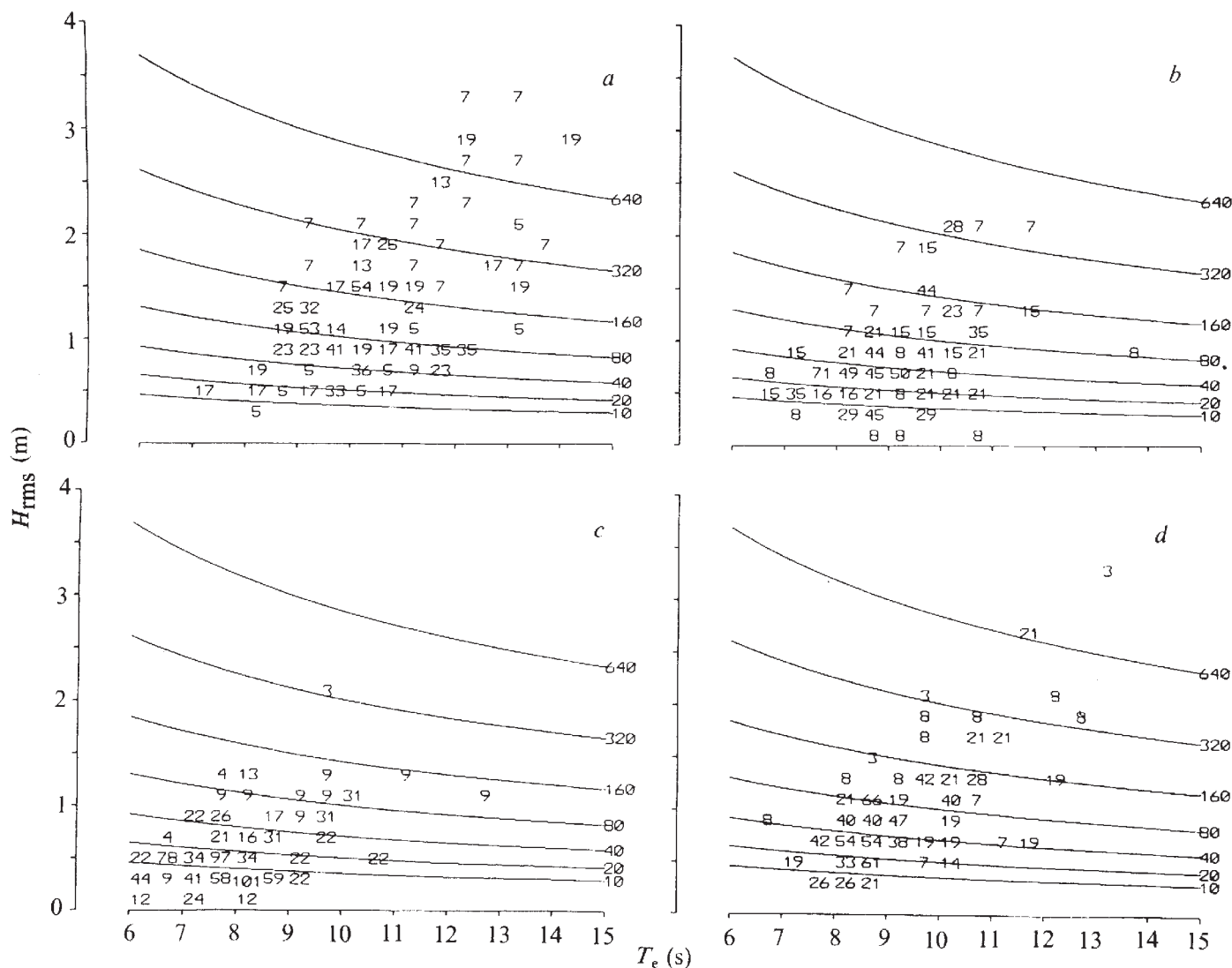
where T_e is called the energy period.

The seasonal joint distributions of T_e and H_{rms} at O.W.S. India are shown in Fig. 1, with contours of P . They are calculated from Hoffman's set of 307 spectra² which were selected from a random sample of just over a thousand 10–15 min wave records, taken over the period 1954–67; we weighted each spectrum to restore the correct frequency of occurrence of each band of windspeed in each season. The overall average power is 91 kW m^{-1} .

The predictions given here are for ducks operating with a simple power output limit and (as in the laboratory) relative to a fixed axis. More realistic physical limits on the torque and angular displacement are important for the estimation of the most economic size and characteristics for full-scale ducks, but should not much affect the range of predicted outputs. The related problems of backbone (axis) movement and the directional wave spectrum are more serious; accurate predictions must await laboratory experiments using a less constrained mounting and data on directional spectra at likely wave power sites.

Figure 2 shows the distribution of power by frequency. (For clarity, autumn and spring are omitted; the former would be close to, the latter rather below, the whole year histogram.) Superimposed are efficiency curves for ducks of diameter $d = 6, 10$ and 16 m , scaled up from the experimental curve (for $d = 10 \text{ cm}$) of Salter, Jeffrey and Taylor³. This curve is the result of attempts to optimise performance at low frequencies, so as to minimise the size of duck required for a given sea. The falling off of efficiency at low frequencies is not unexpected, since it cor-

Fig. 1 Frequency of occurrence at O.W.S. India in % of the possible combinations of T_e and H_{rms} , with contours of P in kW m^{-1} . a, Winter (Dec-Feb); b, spring (Mar-May); c, summer (Jun-Aug); d, autumn (Sep-Nov).



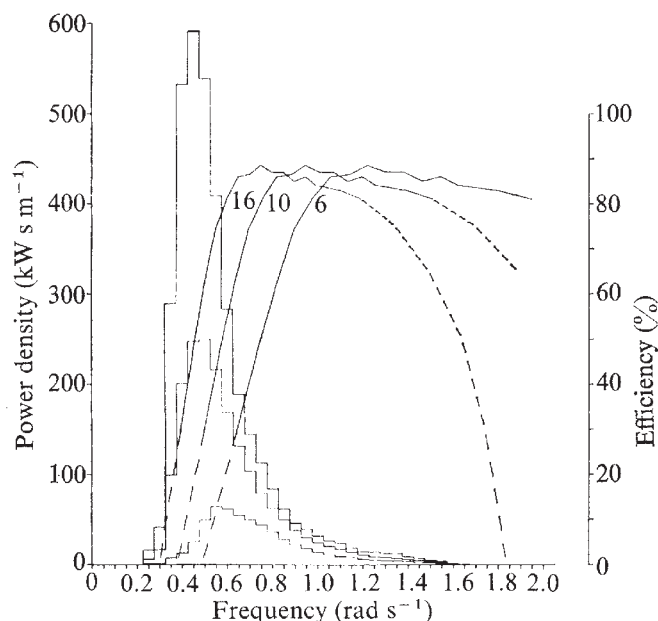


Fig. 2 Distribution of power by frequency for the whole year (middle histogram), winter (top) and summer (bottom); together with predicted efficiency curves for ducks of 6, 10 and 16 m diameter (dashed lines indicate extrapolation outside experimental range).

responds quite closely to the proportion of energy travelling in the water layer above depth d ($= 1 - \exp\{-2d\omega^2/g\}$).

The average output for a particular duck size and season, if no power limits are imposed, can then be found simply by multiplying the appropriate two curves in Fig. 2 and integrating. This is the power transferred from sea to duck; the conversion to electricity and transmission to a distant load-centre in a hydrodynamically underprivileged area, such as South-east England, can be done with presently available technology with an efficiency of $\sim 60\%$. To investigate the variability of power levels, it is of course necessary to refer to the individual spectra. The proportion of time for which power outputs might exceed any given level is shown in Fig. 3, with the total power available for comparison.

We have investigated the effect on power output calculations of replacing the real sea spectra by two-parameter (H_{rms} and T_e) spectra of standard shape, the shape chosen being that of the Pierson-Moskowitz spectrum⁵

$$\int_0^\omega dS = H_{rms}^2 \exp\{-b(\omega T_e)^{-4}\}$$

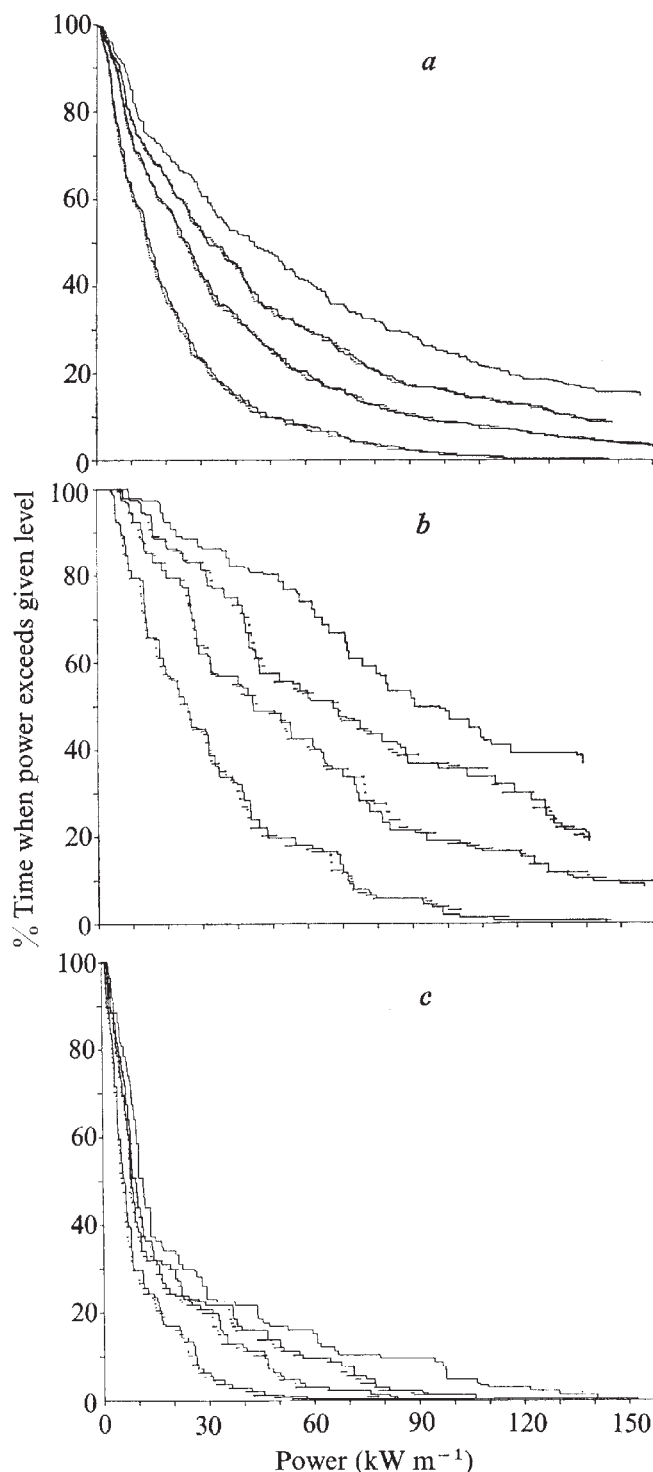
where $b \approx 1,052$ (dimensionless).

This is not only convenient for present computational purposes, but should also greatly facilitate predictions of the effects of physical and torque overloads and the directionality of real seas. This replacement has little effect on the distributions of power input (see Fig. 3, dashed lines), though estimates for individual spectra have s.e. 4.55%. The largest effects on the predicted average output over the range of diameters 6–18 m are -0.5 kW m^{-2} (for $d = 6 \text{ m}$ in the summer) and $+1.5 \text{ kW m}^{-2}$ (for $d = 12 \text{ m}$ in the winter) which may be attributed to real spectra being respectively broader in calm, and narrower in rough conditions than the standard spectra. We conclude that, while further checks are advisable, such standard spectra appear sufficiently accurate for our present approximate predictive purposes. (When directional data become available we intend to extend this investigation to see whether the addition of one further parameter to allow for directional spread yields a similarly adequate description for predicting behaviour of free-floating duck strings in directionally mixed seas.)

Directional problems apart, there are three limitations on output which we have not considered so far: physical (swept-volume), torque and transmission limits. Because of the high costs of transmission lines⁶ the last of these is probably the most critical, though swept-volume overload limits will be important for ducks of diameter $< 10 \text{ m}$.

The mean power available when simple power output limits

Fig. 3 The proportion of time for which each power level is exceeded for: *a*, whole year; *b*, winter and *c*, summer. In each case the top curve refers to the total power available; the other solid curves, in descending order, to predicted power output from ducks of 16, 10 and 6 m diameter. The dashed curves (verticals omitted) show the effect, for each of these three cases, of replacing real spectral data by two-parameter standard spectra (see text).



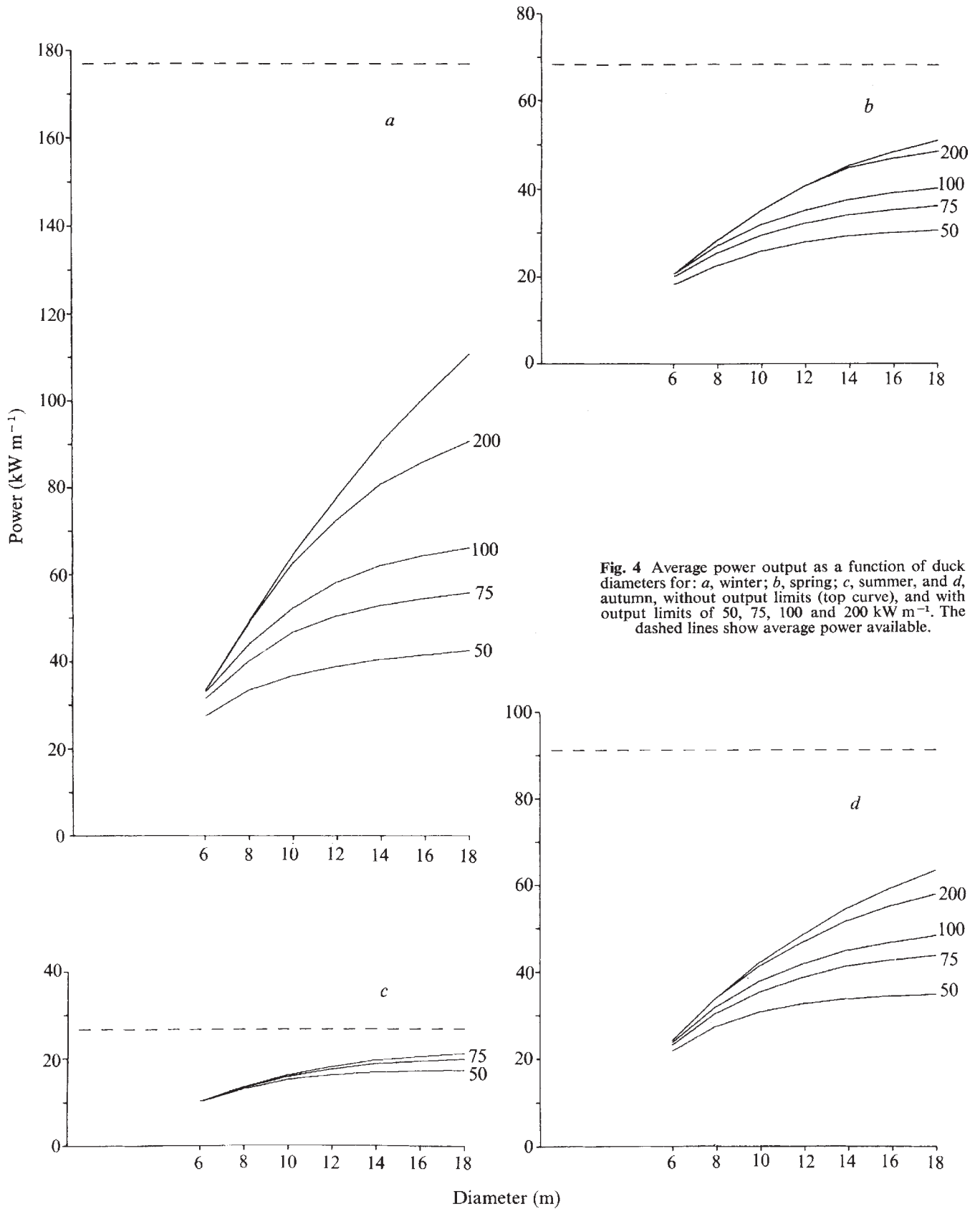


Fig. 4 Average power output as a function of duck diameters for: *a*, winter; *b*, spring; *c*, summer, and *d*, autumn, without output limits (top curve), and with output limits of 50, 75, 100 and 200 kW m⁻¹. The dashed lines show average power available.

are imposed is easily calculable as the area under the relevant curve of Fig. 3 to the left of the desired limit. This is plotted as a function of duck diameter in Fig. 4. As might be expected, the effect of such limits is more severe in winter. Even then the

lowest (50 kW m⁻¹) limit considered gives a substantially higher load factor ($\sim 80\%$ for $d \geq 12$ m), a further reason for preferring a relatively low rated transmission line, rather than going for maximum average output.

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Male emigration and female transfer in wild mountain gorilla

IN many group-living primates, males leave their natal group and transfer to other groups far more commonly than do females (for example, refs 1–4). For only one species—the chimpanzee—is there good evidence that females leave their natal group and transfer more commonly than do males^{5,6}, but little information has been available on the movements in and out of gorilla groups. The structure of the gorilla population in the Virunga Volcanoes of Zaire and Rwanda^{7,8} and elsewhere⁹ indicates that males at least leave their natal group: there are a number of lone males; and many groups contain only one fully adult male, but a number of adult females. Schaller⁹ implied that males transferred between groups more often than did females. The few data in ref. 10 indicate, however, that only females transfer between groups, although both males and females leave groups. Incorporating data for 8.5 yr from the Karisoke Research Centre in the Virunga Volcanoes (ref. 10 and unpublished results), this paper provides the first extensive evidence that both male and female gorillas leave their natal group, although some sons may remain; that males that leave initially travel alone; and that only females transfer.

Groups and lone males will be called 'units'. A distinction must be made between short 'visits' by animals to other units and longer 'transfers'. A 'transfer' is defined as a stay of more than 1 d with the new unit. Males mature when about 11 yr old, their first recorded emigrations being at about this age (Table 1a). Males that did not emigrate were the leading males or animals that were probably some of their sons (Table 1b). Leader males tended to inhibit young males from mating, and competition for oestrous females may be a cause of male emigration. Young males that meet stiff competition will probably leave; those that do not will probably stay, and these are likely to be the sons of ageing leaders. One such male took over leadership of the group when his father died. Such inheritance of leadership may be a pattern common to most groups.

Females become mature at about 8 yr old, and also tended to leave the group at about the age of maturity; they tended to leave before giving birth (Table 2a), as has been reported for chimpanzees⁶. In contrast to males, it seems that nearly all females eventually leave their natal group. Because females transfer between groups, however, for only the four youngest females in Table 2 can we be sure that the group in which they were first seen was their natal group.

The one female that remained in her natal group (Table 2b)

produced an offspring, probably sired by her own father, or by a male who was probably her half-brother. No multiparous females with living offspring emigrated from the group in which they were first observed (Table 2b).

Four males were intermittently observed for 2–5 yr after they first emigrated out of the study groups. None transferred and all spent most of their time alone. Also, no male transferred into any of the study groups. It seems that males that leave their natal group become successful breeders, not by transferring and not by ousting established group leaders (as do langur males^{11,12}), but by attracting more than one female to form a new group. A male and single female seem not to be a viable breeding unit.

Females that emigrate transfer almost immediately to other units (as reported for chimpanzees by Kawanaka and Nishida⁵), and they tend to transfer more than once (Table 2a). The length of stay with one unit before the next transfer ranged from 3 d to 3 yr 5 months, with a median of 3 months ($n = 13$). Initial transfers were to units with ranges overlapping those of the group in which the female was first observed, but subsequent transfers took some of the females out of touch of their initial group's range. Factors that determined whether or not a female stayed with her new unit will be considered later in this paper. Females transferred about equally often to lone males ($n = 11$ transfers) as to groups ($n = 9$ transfers). A number of these transfers were returns to previous units, two of them (possibly 4) to the presumed natal group. If returns are omitted, more females (6 compared with 4) transferred more frequently (9 transfers compared with 4) to more lone males ($n = 4$, possibly 5) than to groups ($n = 2$).

In only three of more than 20 observed potential transfer situations was a male observed actively to prevent a female from approaching the new male, in spite of its being presumably disadvantageous for a male to lose a female that is not his daughter. It seems that males inhibit female emigration more by prevention of proximity of other males than by herding females: of 12 interactions between units during which females transferred, 10 involved intense displays, and sometimes fights, between the males.

It is presumably advantageous to a male, especially a lone male, to gain an unrelated female. Thus, kidnap of females might be expected (Fossey, p. 334 in ref. 13). Out of eight observed visits or transfers, however, only one was possibly caused by coercion by the new male; the others were clearly of the females' own volition. (The discrepancy between the number of interactions (12) and the number of observed visits or transfers (8) is because much of the course of an interaction can be judged from the spoor alone.)

Table 1 Data on males aged 11 yr or more at the end of the study that (a) emigrated from their probable natal group* or (b) remained in it

a	When first observed		When first emigrated		Transfer
	Age	Class	Age	Class	
n					
2	> 12	M	> 14	M	No/No?
1	> 11	M	> 12.5	M	No
1	9.5	Ad	11.5	M	No
1	8	Ad	12	M	No
†1	5.5	J	10	Ad	No?
‡1	2.5	I	11	Ad	No
b	When first observed		When last recorded		
	Age	Class	Age	Class	
n					
2	> 12	ML	> 20	ML	
1	> 12	M	> 20	ML	
†1	5.5	J	13.5	ML	
†1	3	I	11.5	M	

*Because no male transferred between groups.

ML, Mature leader male; M, mature male (> 11 yr); Ad, male aged 8–11 yr; J, juvenile (3–6 yr); I, infant (0–3 yr).

†Almost certainly a son of a leader male; ‡Almost certainly not a son of a leader male. Ages are to nearest 0.5 yr.

Table 2 Data on females aged 8 yr or more at the end of the study that (a) left the group in which they were first seen or (b) remained in it*

a	When first observed		When first emigrated		Parous	Transfer
	n	Age Class	Age Class			
	2	6.5-7 sAd	11, 13 Ad	Yes	Yes	
	2	6-6.5 sAd	9.5, 10 Ad	No	Yes	
	1	5.5 J	10.5 Ad	No	Yes	
	3	1-2 I	6.5, 8, 8 sAd	No	Yes	
b	When first observed		When last recorded		Minimum number of offspring alive	
	n	Age Class	Age Class			
	5	>9 Ad	>17 Ad	1, 2, 2, 3, 3		
	1	1.5 I	9.5 Ad	1		

*This group is almost certainly the natal group of the four infants. Ad, Sexually mature female (>8 yr); sAd, subadult (6-8 yr); J, juvenile (3-6 yr); I, infant (0-3 yr). Ages are to the nearest 0.5 yr.

What factors influence a female's choice of male, and her decision to stay or transfer again? By analogy with ornithological studies¹⁴, and with some data to support the suggestion, the initial decision might depend on the quality of the male's range. Subsequent decisions to stay or transfer might rest on the female's success at raising her offspring in the unit: two mothers who successfully raised their offspring in a new unit stayed; three who did not, left. The infants of at least two of the latter were killed deliberately during interunit interactions.

Finally, why are gorillas and chimpanzees, perhaps the only primates in which females more frequently transfer between units than do males? Four reasons are suggested. The first two are presented in terms of consequences of remaining in the natal group.

● Macaques are the best documented taxon in which males, but rarely females, transfer between groups^{2,3}. Also well documented in this taxon is the phenomenon of offsprings' assumption of agonistic ranks close to that of their mothers', and of females keeping their rank from immaturity into adulthood^{15,16}. Even if immature chimpanzee and gorilla females assumed ranks close to their mothers' (and gorilla females probably do not), there would be little advantage in doing so because of lack of competition in which dominance would be an advantage. Thus, to a gorilla or chimpanzee female, there are less advantages to remaining in her natal group than there are to at least a macaque female.

● Conversely, for chimpanzee males, there are advantages to remaining within their natal community because of communal defence of range and acquisition of females^{6,17,18}.

The final two reasons for female, rather than male, transfer in gorillas and chimpanzees are presented as proximate causes of the observed pattern of, first, emigrations, and second, transfers.

● Although the evidence is diffuse, it seems that young adult males and females tend not to copulate with familiar individuals¹⁹. Given the current state of transfers, females of many, perhaps most, group-living primate species can copulate with unfamiliar mates in their natal group; chimpanzee males can do the same with females; neither gorilla females, nor males (because the dominant male inhibits young males from mating) can do so; and we find that both young male and female gorillas leave their natal group.

● Resident gorilla and chimpanzee males seem more capable than do resident males of other species, of preventing non-group males from entering the group and mating; gorillas, because of small group size and rarity of oestrus; chimpanzees, because of communal attacks on strange males^{17,18}. Thus gorilla males that leave their natal group (no chimpanzee males have been known to do so⁶) travel alone until females transfer to them, whereas in other species, the males themselves tend to transfer.

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Female African wild dogs emigrate

AMONG mammals, the common mechanism of individual transfer between social groups is male emigration. While studying the African wild dog (*Lycaon pictus* Temminck) over a period of 2 yr, we have recorded four positive cases of female group emigration, three possible cases of single female emigration, and only one possible case of male group emigration. From this we conclude that female emigration is the primary mode of interpack transference in African wild dogs. The only other mammal for which this has been reported is the chimpanzee¹.

The African wild dog is a medium-sized carnivore living and hunting cooperatively in packs². Average pack size during our study was 7.2 adults (computed at 3-month intervals for seven packs, an adult being 1 yr and older). Group social organisation in this species usually consists of one breeding pair per pack and a cooperative system of pup rearing by all pack members³⁻⁷. According to zoo records^{7,8} and observations of very small pups at dens^{7,9}, the sex ratio is unequal at birth, with 55-59% males. Of 78 dogs 2 yr and older (and therefore sexually mature) that we studied, 69% were, however, males.

Fieldwork began in January 1974, and is continuing. Individual wild dogs are recognised by their distinct coat colour pattern (Fig. 1). For field identifications, a photographic recognition file is carried. Life histories and family relationships are recorded for all known dogs. These data are augmented by Hugo van Lawick's photographic recognition file for 1968-72. Our study area is 4,200 km² of grasslands and 1,000 km² of surrounding woodlands in northern Tanzania's Serengeti National Park and Ngorongoro Conservation Area. This includes the entire known ranges of four wild dog packs and part of the ranges of two additional

packs. Pack ranges cover a minimum of 1,500 km², and overlap about 10–50% with the range of each of the three or four neighbouring packs. Surrounding our study area there are at least seven more packs. We believe the wild dog population may be depressed by disease. Furthermore, shooting within the park as recently as November 1973 has entirely exterminated some packs.

Previous studies tended to concentrate on one pack of wild dogs, with only infrequent sightings of other packs. Changes in pack composition between sightings were therefore usually impossible to interpret. There are, however, two verified instances and one probable case, of female group emigration from the years 1968–71 (J. R. Malcolm and H. van Lawick, personal communication) in the same area as our study area.

Instances of emigration among our six study packs are shown in Fig. 2. In our study, emigration was verified when the following criteria were met: (1) the original pack was seen alive and intact after the disappearance of some of its members; (2) the missing dogs were seen alive after they had left; and (3) the missing dogs joined a new pack and did not return to their original pack. There were four verified cases of group emigration of females, involving 14 individuals. Four of these females emigrated twice.

Individual females disappeared from their packs in three further cases and were never seen again. Two of these females were sisters, who disappeared individually. Because they might have been expected to emigrate together, but did not, there is some doubt about whether they really emigrated. We believe that both probably did emigrate, however, because all pack members seemed to be healthy just before each sister's disappearance, one of their sisters had established herself as reproductively dominant, and both were of an age beyond which no females remain in packs as non-breeders. The one remaining sister of the dominant female who stayed in the pack was also able to breed. She was, however, clearly subdominant, and was allowed only limited access to her pups. Her mate was probably one of the subdominant males. In the third case, a lone daughter of the pack disappeared. She had no sisters which could have accompanied her, and she was the same age as other young females whose emigrations were verified. Each of these three females probably emigrated and died. Mortality of emigrating lone adult females may account significantly for the preponderance of adult males in the population.

During our study, there was one unexplained dis-

appearance of three male siblings, just at the age of sexual maturity. They probably emigrated from their parent pack, but were not seen again.

In February 1976 we witnessed the emigration of two daughters from the Seronera pack to the three males of the Kühme pack. We followed them continuously for 6 d, which included their departure from the parent pack, their journey in search of the Kühme males, and their subsequent joining. Their emigration seemed to be precipitated by the proximity of the Kühme males who were without a female. The emigrating females tracked the scent trail of these males, and one of them repeatedly made contact calls. At the initial meeting of the two groups, there was a high level of aggressive pawing, shoving, and riding-up between the sexes, initiated by the females (Fig. 1). This behaviour continued for the subsequent 2 d of observation. Within 2 min of meeting, however, it became obvious which male and female would form the new breeding pair. One male excluded the other two males with threats, and one female excluded her sister with threats and attacks. Within 90 min this pair was urine-marking together, in the manner of a breeding pair⁸. Behavioural details of the emigration, and a general discussion of the dynamics of African wild dog packs, and the phenomenon of pack splits, will be published elsewhere⁹.

The following main features of female emigration have emerged in 2 yr of study: (1) Female emigration occurred when a pack already had one breeding female. (2) Emigrating females never joined a pack that already contained a female. (3) Factors which drove or enticed females from their packs are obscure. The suddenness and rapidity of three transfers suggested an outside stimulus precipitating emigration, probably olfactory or visual evidence of a group of males nearby. There was no evidence that oestrus was the initiating factor, although all females were of the age of sexual maturity, and in one case oestrus and conception occurred soon after the females joined new males. (4) Females did not emigrate further than the adjacent or overlapping ranges of neighbouring packs. This was true in all cases, including the group of four females who roamed 4 months before joining new males. (5) Females usually emigrated in groups, if this was possible.

In the existing ecological conditions and low density of African wild dogs, female emigration has been the regular and predictable pattern of interpack transference. Male emigration has been of lesser importance, with no such transfers verified. In packs with offspring of known or

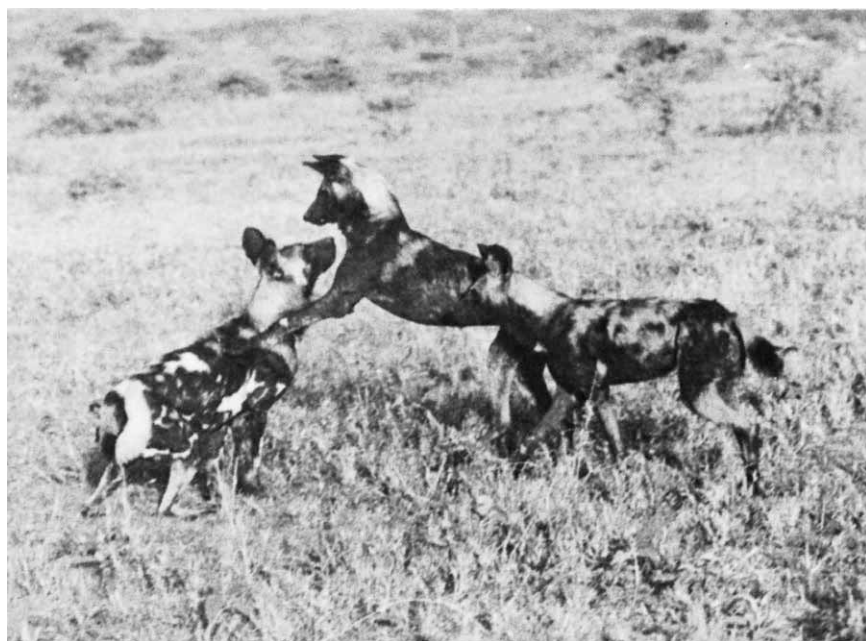
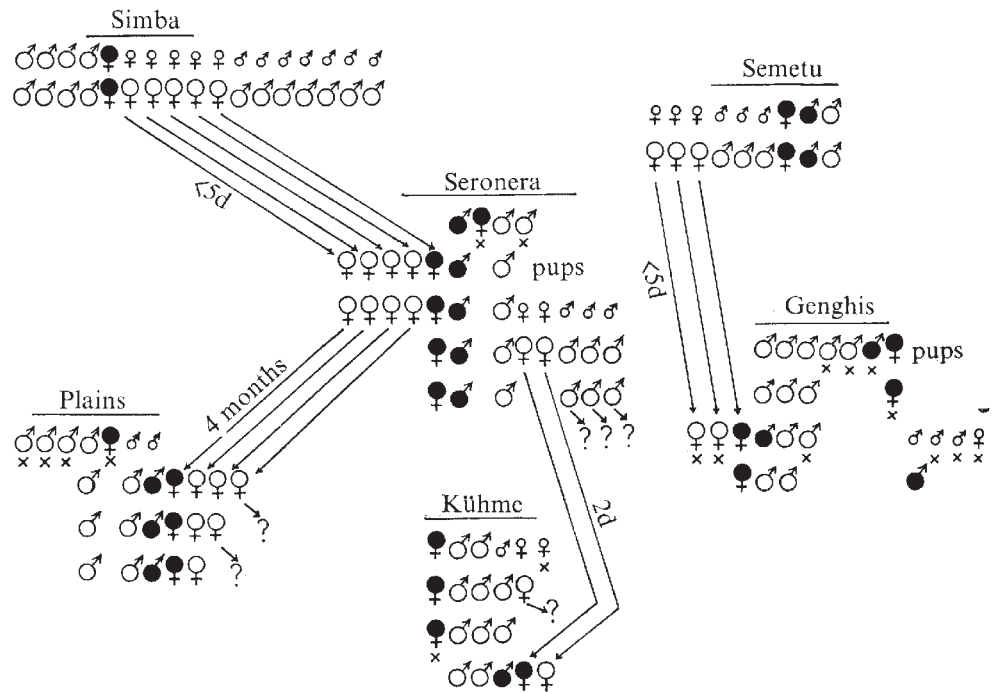


Fig. 1 Side view of three African wild dogs showing distinctive coat patterns used in individual identification. This photograph shows the two sisters (right), who emigrated from the Seronera Pack. During the third morning of their emigration, they met the males of the Kühme pack, who lacked females. A great deal of aggressive pawing, shoving, and riding-up was initiated by the females.

Fig. 2 Diagram of female transference among six study packs of African wild dogs. Three of the four transfers occurred within several days; the fourth took four months. Four females of the Simba pack underwent a secondary emigration after a sister became established as a breeding female in the Seronera Pack. Vertical columns represent the same individual through time. ♂, Adult male; ♀, adult female; ●, dominant breeding pair; ♂, ♀, yearling littermates, ×, dead, ?, disappeared.



reliably estimated age, all daughters left when they were 18–24 months old. Their male siblings usually stayed in the parent pack. A process of secondary emigration existed, whereby sisters of a new breeding female left her after she attained this status. In combination with probable mechanisms which delay some females' breeding, emigration accounts for the usual occurrence of only one breeding female per pack. Differential mortality of females who emigrate singly or in small groups may account for the sex ratio favouring males more in adults than in immature dogs.

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Allometry of neonatal size in eutherian mammals

I HAVE demonstrated that neonatal weight in 15 species of anthropoid primates scales at a power of maternal weight of 0.70 (ref. 1). Gould² noted the closeness of this value to the standard metabolic exponent of 0.75, and asked if this similarity was more than coincidental. In an attempt to answer Gould's query, I have investigated the scaling of neonatal size in eutherian mammals and primates in particular.

Incorporated in this study are data on weights of neonates (foetus at term ± 5 d) and adult, non-pregnant females of 36 eutherian species ('mouse to elephant' curve)³ and 29 primate species⁴, in addition to previously unpublished data on 287 neonates and 316 females of seven species of *Macaca* (*M. fascicularis*, *M. radiata*, *M. nemestrina*, *M. niger*, *M. mulatta*, *M. arctoides* and *M. fuscata*).

Among eutherian mammals and subordinate eutherian taxa, neonatal and maternal weight are allometrically related through the equation

$$y = bx^a$$

where y is neonatal weight (weight of the whole litter if there is more than one offspring), b the initial size constant (y intercept), x the maternal weight, and a the exponent of allometry (slope)^{3,4}. Table 1 and Fig. 1 show that there is a concomitant change of a and taxonomic level. This situation parallels the finding for the brain size–body size relationship⁵. On the other hand, for the scaling of the other principal organs (for example, heart, lungs and liver), the relationship between a and taxonomic level is far less regular^{6–8} (Table 1 and Fig. 1).

An assessment of the various values of a with taxonomic level requires a causal explanation. In the scaling of brain size, the combined influence of differences in the evolutionary level of brain development and differences in mean body size of groups at the same taxonomic level accounts for differing values of a (ref. 2). Similarly, in the scaling of neonatal size, differences in the structure and physiology of foetal membranes and placenta in combination with differences in mean body size must be considered. Thus, a meaningful interpretation of a

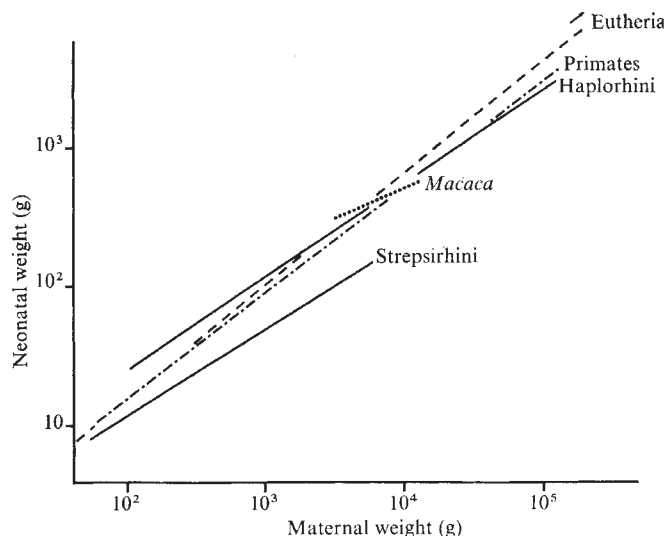


Fig. 1 Neonatal-maternal weight relationships in Eutheria, Primates, Strepsirhini, Haplorhini and *Macaca* (based on values from Table 1).

in the scaling of neonatal size requires that either corrections are made for structural and physiological dissimilarities during foetal development, or comparisons are limited to taxa with members that are structurally and physiologically similar.

Among the Eutheria basic similarities in foetal membrane development only exist during early blastocyst formation⁹, after which there is considerable variation in the development of foetal membranes and placenta among the higher taxonomic categories (orders and suborders)⁹. Because it is virtually impossible to quantify the influence of structural and physiological differences in foetal development on neonatal size for a taxon as diverse as Eutheria, a meaningful interpretation of a is difficult. In any case, a (0.83) for Eutheria is clearly above the standard metabolic exponent.

Primates are also variable in the foetal development of many features. For example, there is a strong dichotomy in the structure of the chorio-allantoic placenta and associated foetal membranes. The members of the suborder Strepsirhini have a non-invasive, diffuse, epitheliochorial placenta, a large allantois and a transitory chorio-vitelline placenta, whereas an invasive, discoidal, haemochorial placenta and rudimentary allantois characterise members of the suborder Haplorhini⁹. Although the standard metabolic exponent (0.75) is within a 95% confidence interval of a (0.78 ± 0.05) for Primates, this similarity may be merely coincidental. Actually, the value of a in Primates is due to the combined result of lower but similar values of a in the two suborders, the separation of the two suborders by an allometric shift (differing values b due to differences in foetal membrane and placental structures), and a difference in mean body size between the two suborders.

In contrast to the infraclass Eutheria and the order Primates, structural and functional similarity in foetal membranes and

placenta exists within each of the suborders Strepsirhini and Haplorhini. It is interesting that, whereas the standard metabolic exponent (0.75) lies outside the 95% confidence interval of a for both Strepsirhini (0.63 ± 0.05) and Haplorhini (0.69 ± 0.04), another 'standard' exponent (0.67) is within these confidence limits. The value of 0.67 is known as the standard exponent for the interspecific ordinal scaling of brain size. It implies a determination of brain weight by body surfaces rather than by volume, a finding that has not yet been satisfactorily explained². Similarly, it is difficult to explain the geometric similarity between neonatal weight and maternal surface. One explanation may be that, assuming (as for other organs⁵) virtual isometry of placental weight and maternal weight, placental surface rather than volume determines neonatal weight.

Among macaque species, a (0.49 ± 0.03) is clearly below 0.75 and 0.67. As with other aspects of scaling of neonatal size, a parallel situation exists in the scaling of brain size. Studies of brain-body size relationships in primates¹⁰⁻¹³ have shown that brain weights in adults of a single species (or a cluster of related species so similar that they appear as different size expressions of the same body plan) scale at a much lower power of the body weight (generally between 0.2 and 0.4) than more distantly related species. As with intrageneric curves of brain size², the present findings on macaque species suggest that the low value of a in an intrageneric curve of neonatal size is probably a consequence of the intraspecific pattern which in turn represents the correlated variability on which selection acts.

In summary, the findings on neonatal-maternal weight relationships in eutherian mammals indicate that neonatal size is not scaled metabolically. In taxa that are structurally and functionally equivalent in foetal membranes and placenta, neonatal weight scales at a power of maternal weight close to 0.67. This suggests that maternal (placental) surfaces rather than volume determine neonatal size.

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Table 1 Scaling of neonatal weight in eutherian mammals and subordinate eutherian taxa

Taxon	Level	N	b	a	μ	r
Eutheria	Infraclass	36	0.34	0.83	—	0.92
Primates	Order	29	0.41	0.78	0.05	0.97
Strepsirhini	Suborder	9	0.64	0.63	0.05	0.99
Haplorhini	Suborder	20	1.08	0.69	0.04	0.99
<i>Macaca</i>	Genus	7	6.22	0.49	0.03	0.97

N, Sample size (number of species); b, initial size constant (y intercept); a, allometric exponent (slope); μ , 95% confidence interval of a; r, correlation coefficient.

—, No value given in literature.

Phenotypic variability of inbred and outbred mice

THE relative phenotypic variability of inbred and commercially available outbred stock mice has never been satisfactorily investigated, even though it has important implications in the design of animal experiments. Early workers¹ suggested that "the published facts suggest that inbred strains are usually more variable than inter-strain hybrids, with random bred colonies occupying an intermediate position." Later it was concluded that with respect to phenotypic variability, "no

general law can be asserted on the comparison of inbred strains with randombred stocks^{7,8}. A subsequent debate on the suitability of inbred, outbred and F_1 hybrid mice for biological assay³⁻⁶ failed to clarify the situation, and many research workers were left with the impression that inbred animals are more variable than outbred ones. Much of the early work could be criticised on the grounds that sample sizes were small, and few populations were sampled. We have collected extensive data on the phenotypic variability of the shape of the mandible, as part of a routine genetic quality control programme^{7,8}, and have found that assuming equal sensitivity to an experimental treatment 50% more outbred than inbred mice would be required to detect a 1-unit change in mandible shape.

Samples of mandibles from inbred (I), outbred (O), F_1 (F_1) and F_2 (F_2) hybrid mouse colonies were obtained from a number of locations and colonies, as summarised in Table 1.

The outbred stocks were named as: TO, BKW, CFLP, LACA, LACG, ICI, MF1, CD-1, CFW, Porton and CF₁. They were all commercially available "white mice" (except for the LACG) typical of those used for many research purposes. Inbred strains included the following, A, A2G, AKR, B10.BR, B10.A, B10.LP-*a*, BALB/c, C57BL, CBA/Ca, CBA/H-T6, CFN1H, C3H/He-*mg*, C3H/He, CE, C57BL/10, C57BR/cd, DBA/1, DBA/2, F/St, ICFW, NZW and 129/RrJ. The F_1 hybrid mice were (C3H × DBA/2) F_1 and (BALB/c × C57L) F_1 . The F_2 hybrids were (NZB × NZW) F_2 and (BALB/c × C57L) F_2 .

Each sample consisted of 8–28 (average 12) male mice over 6 weeks old, and usually weighing 25–30 g, and at least two separate samples were obtained from each colony.

Mandibles were prepared and measured as previously described⁹ using 11 measurements per mandible, and the shape of each mandible was described by two discriminant functions

$$d_i = \sum_j \beta_{ij} X_j \quad \begin{matrix} i = 1, 2; \\ j = 1, 2, \dots, 11 \end{matrix}$$

where the β_{ij} are a set of constants given elsewhere (ref. 8, Table 3) calculated from a previous set of data (not included in this study) and the X_j are the individual measurements expressed as a percentage of the sum of the measurements, in order to

Classification	F_2	O	I	F_1	Total
Locations	1	7	5	5	—
Colonies	2	14	28	2	—
Named stocks	2	11	22	2	37
Samples	10	63	107	14	194
Mice	114	873	1,101	256	2,344

correct for variation in size of individuals. The traits d_1 and d_2 may be regarded as numerical indices of mandible shape corrected for size. The data analysed in this study are the within-sample standard deviation of the two discriminant functions d_1 and d_2 , designated S_1 and S_2 in the 194 samples from the four different classes of stock. Although d_1 and d_2 discriminate well between strains, they do not discriminate between I, O, F_1 and F_2 classes, and there is no evidence for any association between mean and variance. Thus, the use of these two functions should not introduce any bias when comparing the four classes of stock. Also, d_1 and d_2 are uncorrelated.

Assuming that the two characters d_1 and d_2 have a polygenic mode of inheritance, the variation within the F_1 crosses should give a direct estimate of the environmental variance V_E . The total genetic variance (including additive and non-additive components) within the F_2 and O classes of stock may then be estimated by subtraction.

The distribution of the within-sample standard deviations is shown in Fig. 1 for trait S_1 . Within the inbred class of stock there was no evidence that some strains were more variable than others. Similarly, there was no evidence that some of the outbred colonies were more variable than others, though Fig. 1 shows that the variation between samples was greater than with the inbreds, and as some are likely to be more inbred than others, differences could have been expected.

Pooling the data within each class of stock, the following relationships for phenotypic variability were established

Trait S_1 : $F_2 > * O > ** I > F_1$

Trait S_2 : $F_2 > * O > ** I > F_1$

*Significant ($0.01 < P < 0.05$)

**Significant ($P > 0.01$)

The mean phenotypic standard deviation in each class of stock, together with standard errors, is summarised in Table 2. Inbreds were slightly but not significantly more variable than F_1 hybrids, but were substantially less variable than the outbreds, which in turn were less variable than the F_2 hybrids.

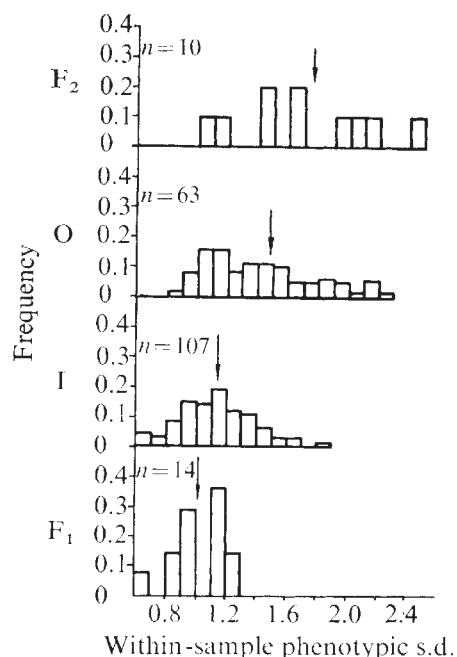


Fig. 1 Distribution of the within-sample phenotypic standard deviation of an index of mandible shape in F_2 hybrid, outbred (O), inbred (I) and F_1 hybrid mice. Arrows indicate the mean, n is the number of samples. Sample size averaged 12 mice.

Table 2 Mean phenotypic standard deviation (and its standard error) of two indices of mandible shape ($\bar{S}_1$ and $\bar{S}_2$)

Class of stock	No. of samples	$\bar{S}_1$	s.e.	$\bar{S}_2$	s.e.
F_2	10	1.73	0.161	1.52	0.101
O	63	1.49	0.055	1.39	0.050
I	107	1.14	0.022	1.06	0.030
F_1	14	1.01	0.043	0.97	0.056

Estimates of the environmental variance, and the genetic variance within the F_2 and O stocks is given in Table 3. Heritabilities (in the broad sense) of d_1 and d_2 were estimated as 0.66 ± 0.175 and 0.59 ± 0.216 , respectively. As single-cross F_2 stock has a coefficient of inbreeding of 0.5 (ref. 10), the relatively low genetic variance of the O stock compared with the F_2 stock implies a substantial degree of inbreeding, probably equivalent on average, to four or more generations of brother × sister mating.

This study shows unequivocally that for these two highly heritable polygenic characters outbred stocks are substantially more variable than inbreds. The implications in terms of number of animals required in an experiment may easily be

Table 3 Genetic and environmental variances

Variance	Estimated from	Trait S_1	s.e.	Trait S_2	s.e.
V_E	V_{F_1}	1.02	0.061	0.94	0.076
V_G	$V_{F_2} - V_{F_1}$	1.97	0.398	1.37	0.230
$V_{G_0}^{F_2}$	$V_0 - V_{F_1}$	1.20	0.129	0.99	0.124

calculated on the assumption that none of the classes of stock is likely to be more sensitive to an experimental treatment than another. In these circumstances, an experiment set-up to detect a 1 unit change in mandible shape between treated and control groups (assuming type 1 and 2 errors are set at 5%, and 10% respectively) would require 13 F_1 , 16 I, 25 O or 34 F_2 animals. Thus, in this case, more than 50% more outbred than inbred animals would be required, though the difference would not be so large in the case of characters with a lower heritability.

These findings give added support to the case for the more widespread use of inbred strains¹¹.

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Fine structural changes in human astrocyte carrier lines for measles virus

CHRONIC infection with measles virus is causally related to the human syndrome of subacute sclerosing panencephalitis (SSPE)¹⁻⁵, and means of reproducing that condition in experimental animals are being sought⁶⁻¹⁰. Measles carrier states *in vitro* have been established in some human¹¹⁻¹³ and rodent^{14,15} cell systems. We now report the establishment of measles carrier states in human astrocyte cultures of normal brain, and in a tumour astrocyte line, as well as the emergence of fine structural features which bear a close resemblance to findings in the brain of some patients with SSPE¹⁶.

Two strains of measles virus were used—wild type (WT) derived from a pharyngeal swabbing of a patient with measles, and Edmonston (ED), a laboratory strain¹⁷. Infectivity (TCID₅₀/0.1 ml of cell-free supernatant), haemagglutination (HA) and neutralisation titrations followed published procedures¹⁷⁻¹⁹, as did primary and secondary cell culture techniques²⁰. The same batches of Eagle's MEM and of foetal calf serum were used throughout.

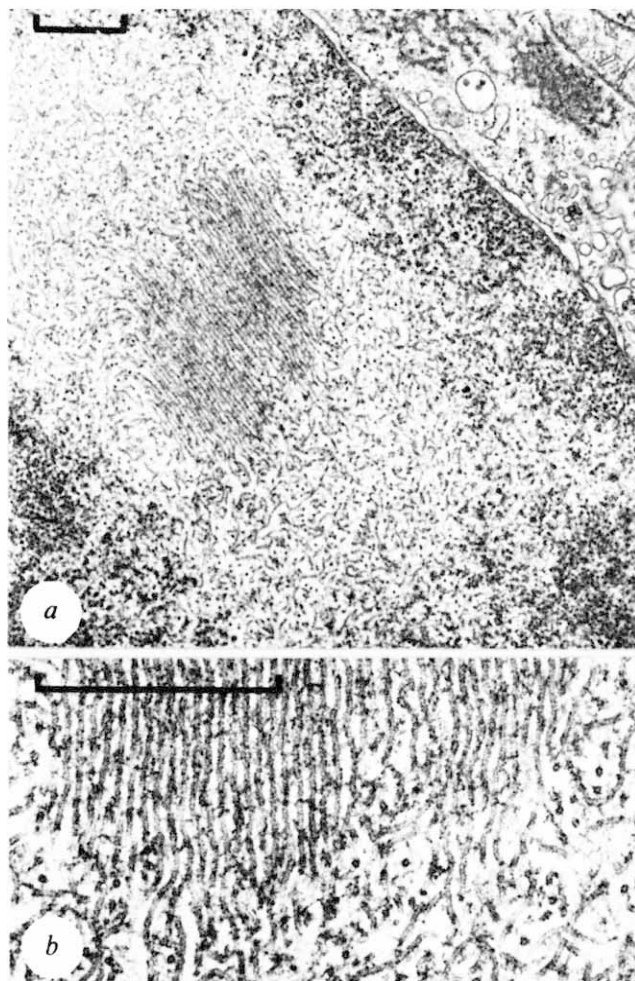
In initial work to establish measles carriers we used the IN1 tumour astrocyte line²¹; equivalent IN1 cells had been used successfully to initiate the V-IN1 line of visna virus-producing human astrocytes²². Subsequent work with astrocytes derived from two normal foetal brains (HFA1, HFA2) and with WT virus gave an essentially similar pattern of virus-cell interaction. The following description of the establishment of the ED-human tumour astrocyte line (ED-IN1) will therefore serve, save for minor variations of time scale, for the ED carriers derived from normal astro-

cytes (ED-HFA1; ED-HFA2) and the WT astrocyte carriers (WT-IN1; WT-HFA1; WT-HFA2).

IN1 cells at passage 125 were used. At this passage level, the cells contain glycogen²¹, synthesise high levels of adenyl cyclase²³ and respond appropriately to catechol stimulation²⁴, as befits astrocytes. IN1 cultures at a density of 1×10^4 cells per mm² were exposed at 37 °C to 0.01 TCID₅₀ ED virus per cell for 2 h, then rinsed and returned to growth medium. Binuclear cells were plentiful in 2 d, small syncytia in 3, huge syncytia in 5; cytolysis began on about day 5 and was almost total by day 9. Intracellular and cytoplasmic inclusions were made visible by Giemsa staining. Infectivity titres at day 7 after infection were $10^{4.5}$ TCID₅₀ and HA was 14-28. These results are in accord with an acute cytolitic infection of classical type by measles virus^{17,18}.

In occasional cultures, scattered cells survived for 14 d; such survivors were likely to develop into carriers. The ED-IN1 line was derived from cells infected as above: a few discrete cells survived for 9 d after infection; these were elongated and had prominent paranuclear vacuoles. At 14 d after infection five small cell groups were present; 25 d after infection mitoses were frequent; the most common cell was plump and fusiform. Small syncytia appeared 31 d after infection among the other cells, grew slowly to maximum size (always much smaller than in cytolitic states) in 8 d and eventually rolled off, while other syncytia developed. The predominant cell was mononuclear. At 60 d after infection the cells were trypsinised for the first time, and designated

Fig. 1 a, Intracellular inclusion, as seen in cytolitic cultures of IN1 cells 5 d after infection with ED measles virus. Higher magnifications (b) show them to consist of arrays of 16-nm smooth nucleocapsid tubules. From fourth transfer onwards, such tubules were not observed in the carrier lines. The bar represents 500 nm.



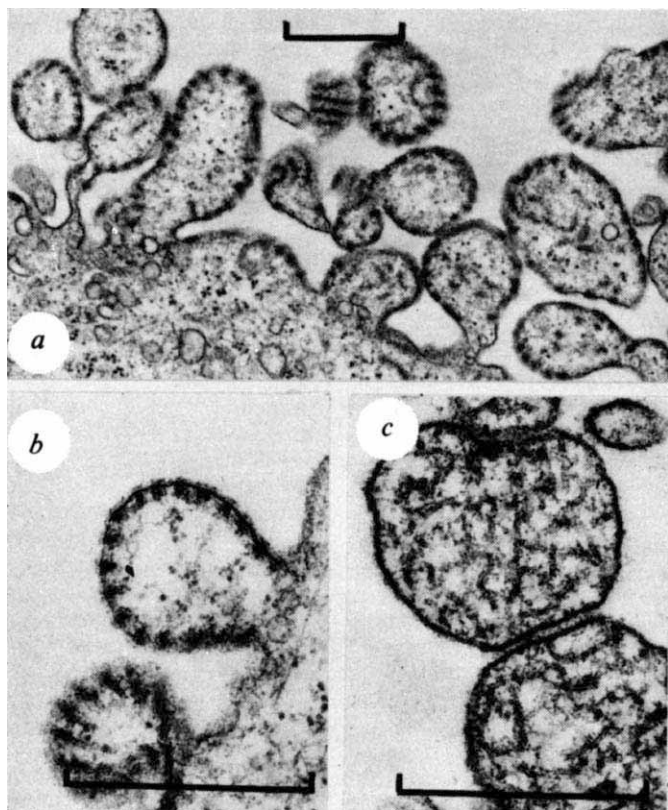


Fig. 2 *a* and *b*, Development and structural detail of complete measles virions at the cell surface, as found in both cytolytic and carrier cultures. *c*, Structurally defective particles observed (beside complete virions) from fifteenth transfer stage in the carrier lines. Note the lack of external projections and disorderly packaging of the nucleocapsids. The bar represents 500 nm.

ED-1N1². Intranuclear and cytoplasmic inclusions were present in both fusiform cells and syncytia. Infectivity titres at this stage were 10^5 TCID₅₀. Between transfers 5 and 15 they were consistently 10^5 TCID₅₀, while HA titres were 28–56 and anti-measles neutralisation stood at 1/4. In later transfers, interference was evident, that is, no syncytia were expressed at 10^0 , 10^{-1} and $10^{-1.5}$, but they were seen at high dilutions of virus. Morphologically atypical virus was detected subsequently in electron micrographs of such cultures. So far the ED-1N1 line has had 21 trypsinisations; this covers 32 weeks from initial trypsinisation. The cells continue to grow well, to produce inclusions and scattered syncytia, and virus titres now stand at $10^{3.5}$ TCID₅₀. The HA titres of the WT lines (WT-1N1; WT-HFA1; WT-HFA2) are 128–256; those of the ED lines (ED-1N1; ED-HEA1; ED-HEA2) are 14–28. The WT lines are at transfer 12.

For electron microscopy^{21,22} representative cultures of the 1N1 cells 5 d after infection with ED strain and from transfers 3 to 21 of the noncytolytic ED-1N1 carrier line were compared with equivalent transfers of uninfected HFA1, HFA2 and 1N1 cells, and with WT-1N1^{6,12}, WT-HFA1⁶, WT-HFA2⁶ and ED-HFA1⁶ carrier lines.

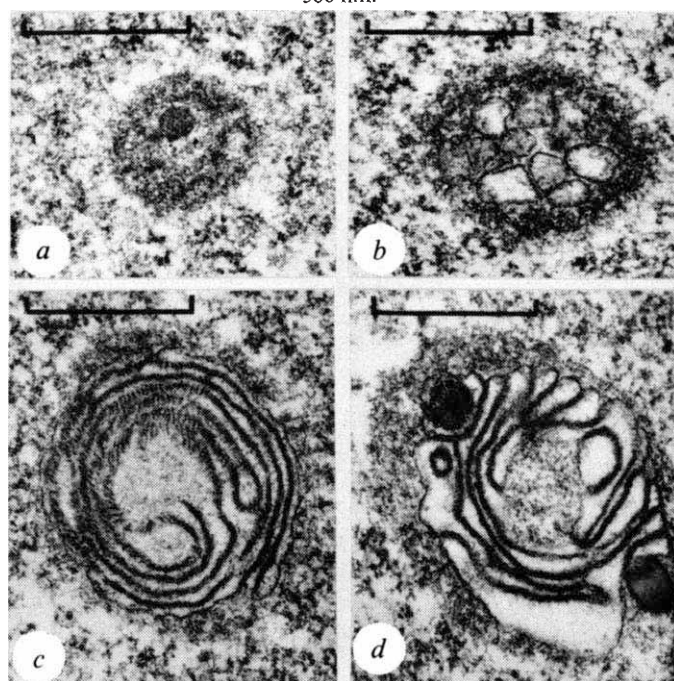
The ultrastructure of the cytolytic cultures showed all the usual intranuclear and cytoplasmic features (see for example, refs 26 and 27) (Fig. 1*a* and *b*). Extracellular virions featured a surface layer of 8-nm projections, characteristically thickened envelope membrane, and nucleocapsid tubules disposed peripherally in spiral form (Fig. 2*a* and *b*). The infected nuclei also had many rounded condensations of microfilamentous material resembling the “nuclear bodies” of normal cells²⁸.

Between transfers 3 and 21 of the ED-1N1 carrier line there were three morphological changes: (1) the dis-

appearance of measles intranuclear nucleocapsids, (2) the appearance in the nuclei of complex structures seemingly derived from nuclear bodies and (3) the emergence of a proportion of morphologically defective virus particles at the cell surfaces. Intranuclear aggregates of measles nucleocapsid^{26,27} were not found in any of the carrier cultures. In rare cells at transfer three scattered tubular profiles were found whose size matched that of smooth nucleocapsid. From the fourth transfer onwards, even these occasional structures were absent. At this stage, however, unusually large nuclear bodies (up to 800 nm diameter) became frequent, some with centrally placed clusters of vesicles (Fig. 3*b*). By transfer 7, enlarged, structurally abnormal nuclear bodies, from 500 nm to 3.5 μ m in diameter and with a well developed membranous component, were a feature of almost all the cells. Four types could be recognised, perhaps representing different developmental stages: (1) simple microfilamentous, (2) vesicular, (3) complex laminar and (4) cisternal (Fig. 3*a–d*). A single nucleus often held different types. Such bodies were invariably demarcated from surrounding nuclear structures by a peripheral zone of microfilamentous matrix, like that of nuclear bodies in control cells²⁸. The new structures were easily distinguished from nucleoli or infoldings from the nuclear envelope. Most striking were the large laminar and cisternal types, in which paired membranes formed closely coiled profiles, with various degrees of focal distension or ballooning. A layer of fine (9–10 nm) regularly spaced filaments often was intercalated between the paired membranes (Fig. 3*c*). Such modified nuclear bodies, and an absence of measles intranuclear nucleocapsid tubules, also characterised the other carrier lines. Extensive surveys of uninfected cultures showed normal small nuclear bodies; no suggestion of the more complex structures was ever seen.

All carrier lines had numerous, large cytoplasmic inclusions akin to those found in cytolytic cultures. Virus particle morphogenesis was evident in all carriers; but in later transfer stages, especially the 15th and 21st transfers

Fig. 3 Four types of nuclear bodies, present within nuclei of ED-1N1 carrier cultures at fourth and seventh transfer stages: *a*, simple microfilamentous; *b*, vesicular; *c*, complex laminar; *d*, cisternal. The more elaborate forms were a prominent feature from the seventh transfer stage onwards. The bar represents 500 nm.



of the ED-1N1 line, other types of membrane-bound particles were also found (Fig. 2c); their envelopes resembled unmodified host plasma membrane, lacking external projections or thickening of the unit membrane. The contained nucleocapsid tubules lacked the characteristic peripheral and spiral disposition of complete measles virions (Fig. 2a and b). These were considered to be morphologically defective measles particles: their presence would seem to complement the observation of viral interference noted in infectivity titrations.

We conclude that the measles carrier lines exhibited a common pattern of fine structural changes, whether derived from normal or tumour astrocytes and whether infected with ED or WT virus. It would be interesting to know whether measles vaccine preparations can induce a similar *in vitro* response in suitable host cells. A prominent feature of the carrier lines, not reported previously even in long term measles-infected organ cultures¹⁴, was the emergence of complex intranuclear formations interpreted as modified nuclear bodies. Martinez *et al.*¹⁶, in a review based on eight autopsied cases of SSPE, defined a category of nuclear inclusions believed to be of diagnostic significance and representing altered nuclear bodies, distinct from paramyxovirus-like nucleocapsid tubules also reported in this disease^{1-5,26,27}. Enlarged nuclear bodies of the same kind were in evidence from the third transfer onwards in our astrocyte carrier lines, together with even more elaborate forms with a well developed membranous component. Simple hypertrophy of nuclear bodies is well known in measles, and some other virus infections²⁸, but their relationship, if any, to synthesis of specific viral components is still largely speculative. SSPE is associated with a defective form of measles virus⁴. Although the carrier lines continued to produce infective virus throughout, evidence of defective particle production was also obtained.

Functional and structural features of the measles carrier state in human astrocytes seem therefore to present interesting parallels with the disease SSPE.

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Environmental carcinogens and large bowel cancer

THE geographical variation in the incidence of large bowel cancer together with the increased incidence found in migrants from low risk to high risk areas support the view that environmental factors are involved in the aetiology of this disease¹. These environmental factors are thought to be largely dietary although other influences have not been excluded. Aries *et al.*² suggested that the disease is caused by carcinogens produced by gut bacteria from bile acids. Diet would influence both the bile acid levels in the bowel and also the nature of the gut flora. Faecal bile acids are more concentrated in faeces from people living in high incidence areas than in faeces from populations at low risk³. Similarly, faecal bile acids are usually more concentrated in samples from patients with large bowel cancer than in those from control patients⁴. Bile acids were postulated to be the substrates from which the gut flora produced carcinogens² and indeed this suggestion has been supported by the demonstration that the faecal concentration of bacterially degraded bile salts is more highly correlated with the incidence of large bowel cancer than is the total bile acid level⁵. However, it has been suggested that the primary role of bile acids is as co-carcinogens and this suggestion is supported by the results of animal experimentation demonstrating the promoting effect of bile acids on the action of known carcinogens⁶. If it is accepted that bile acids act as co-carcinogens it becomes necessary to explain how the carcinogen reaches the large bowel without producing cancers in other parts of the gastrointestinal tract. Here we describe preliminary investigations of a mechanism by which an environmental carcinogen could be transported to the large intestine.

The conjugates and metabolites of benzo[a]pyrene were prepared biologically. This compound has been shown to undergo oxidation and conjugation in the rat and is excreted mainly in the bile as glucuronic acid and sulphate conjugates of phenols, as conjugates of dihydrodiols and possibly as mercapturic acid conjugates^{6,7}. Two bile duct cannulated female Wistar albino rats were given 7,10,14-¹⁴C-benzo[a]pyrene (Radiochemical Centre, Amersham); 1 mg; 14 μ Ci per rat. The bile was collected for 4 h and found to contain 6.7 and 12.5% of the administered ¹⁴C. Analysis by thin layer chromatography⁸ showed a single peak of radioactivity at the origin, corresponding to conjugated metabolites. Aliquots of the pooled bile containing the ¹⁴C metabolites of benzo[a]pyrene were incubated with samples of human and rat faeces homogenised in nutrient broth. Further aliquots of the bile were incubated with pure cultures of bacteria representing the major types found in the intestine. After incubation for 72-96 h under strictly anaerobic conditions the reaction mixtures were analysed by thin layer chromatography.

Almost all the bacteria tested were able to hydrolyse the conjugates to release the oxidative metabolites of benzo[a]pyrene (Table 1) and the major conjugated metabolites of benzo[a]pyrene seem to be phenols and quinones. It is, however, possible that further metabolism of the unconjugated metabolites may have occurred, and these phenols may have been produced from or converted to dihydrodiols and, to some extent, to benzo[a]pyrene. Of particular interest are the findings that the organism releasing the greatest amount of the parent hydrocarbon in this test system, *Clostridium paraputrificum*, is present in larger numbers in the faeces of "western" subjects⁹ than in faeces from low risk areas, and the *Bacteroides* spp. show this activity as well as deconjugation of taurocholate and dehydroxylation of bile acids.

Table 1 The bacterial hydrolysis of the conjugates of benzo[a]pyrene excreted in the bile of rats

	Conjugates hydrolysed (%)	Proportion of benzo[a]pyrene and its metabolites		
		Dihydrodiols	Phenols and quinones	Benzo[a]pyrene
Rat faeces	28.0	15.9	11.3	0.7
Human faeces	28.4	10.2	17.1	0.9
<i>B. fragilis</i> 9343	14.6	2.2	11.8	0.8
<i>B. fragilis</i> ST 2	26.0	7.2	18.4	0.4
<i>Cl. perfringens</i> 10379	12.6	1.9	10.4	0.3
<i>St. faecalis</i> 775	13.5	2.6	10.5	0.4
<i>E. coli</i>	10.0	3.1	6.8	0.1
<i>Kl. pneumoniae</i>	13.2	2.0	10.5	0.7
<i>Cl. sporogenes</i>	8.9	0	8.9	0.3
<i>Cl. sporogenes</i>	0	0	0	0.3
<i>Cl. parapsitricum</i>	12.3	0.1	11.4	0.9

The results are expressed as the percentage of the total radioactivity in the incubate.

Rat bile (100 µl) containing the conjugated metabolites of benzo[a]pyrene was incubated at 37 °C under anaerobic conditions for 72–96 h. Incubation in the absence of microorganisms showed a limited hydrolysis and conversion to benzo[a]pyrene, that is 10.0 and 0.5% respectively, which has been subtracted from the bacterial incubations to give the above results.

These preliminary experiments show that bacteria of the types found in the human intestine are able to hydrolyse the conjugated metabolites of benzo[a]pyrene excreted in the bile of the rat. A small, but significant, amount of benzo[a]pyrene was also released, probably as a result of the dehydroxylation of hydroxylated metabolites. These reactions are a reversal of the detoxication processes performed by the liver. An analogous mechanism is thought to explain the intestinal cancers produced in rats as a result of the administration of 3-methyl-4-aminobiphenyl and 3-methyl-2-aminonaphthalene, and the relevance of such studies with aromatic amines to large bowel cancer was reviewed by Weisburger¹⁰.

Polycyclic aromatic hydrocarbons, such as benzo[a]pyrene, are widely distributed in nature and arise mainly from the combustion of organic matter, especially fossil fuels. Their concentration in the environment is thus increased by industrial activity. Engst and Fritz²¹ showed concentrations of 0.8 µg kg⁻¹ in sea sand, up to 0.8 mg kg⁻¹ in rural soils but 65 mg kg⁻¹ in the industrial urban environment. These compounds also occur in smoked foods. We suggest that polycyclic aromatic hydrocarbons such as benzo[a]pyrene ingested from the environment may act as the primary carcinogens in the development of large bowel cancer. The geographical variations in the incidence of this disease could then be partly explained in terms of the variations in the distribution of the carcinogens associated with industrial activity, since the incidence of colon and rectal cancer is highly correlated with gross national product¹².

The consumption of a western diet, rich in fat and protein, is undoubtedly also important. Diet probably influences the metabolism of polycyclic aromatic hydrocarbons directly, as a result of its influence on mucosal enzymes and indirectly by the effect on bile acid metabolism. Aryl hydrocarbon hydroxylase activity is present in the duodenal mucosa of rats fed fat-containing diets and may be induced by exposure to benzantracene. The enzyme is present at very low levels in the mucosa of rats fed fat-free diets and is detected in the colon of rats fed fat-containing diets only after exposure to benzantracene¹³. The levels of other enzymes, associated with benzo[a]pyrene metabolism in the gut wall, have not been extensively investigated.

There are two ways in which bile acids may be important in the metabolism of polycyclic aromatic carcinogens excreted in the bile. (1) The conjugated metabolites of benzo[a]pyrene in the bile are amphipathic compounds, as are the bile acids. An important function of bile acids is the formation of mixed micelles during fat digestion and it is possible that polar conjugates are incorporated into micelles thus aiding their transport through the small intestine. (2) Polycyclic aromatic hydrocarbons are lipid soluble and it has been suggested that this property would render them inactive in the aqueous environment of the gut¹⁴. Bile acids are,

however, powerful solubilising agents and would maintain the carcinogens in solution and aid their interaction with cell surfaces.

In conclusion, we would suggest that large bowel cancer results from the retoxification by the gut flora of the liver metabolites of polycyclic aromatic hydrocarbons. This process is influenced by the effect of diet on the intestinal cells and secretions, particularly bile acids and possibly by the effect of diet on the gut flora. The disease is more common in western countries because (1) industrial activity increases the amounts of polycyclic aromatic carcinogens in the environment and (2) the western diet favours the retoxification process.

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T–T cell collaboration during *in vivo* responses to antigens coded by the peripheral and central region of the MHC

Mixed lymphocyte culture (MLC)¹ has been used extensively as an *in vitro* model to analyse the reactivity of T cells to antigens coded by the major histocompatibility complex (MHC). When murine T responder cells are exposed *in vitro* to allogeneic lymphoid cells (stimulator cells) they proliferate and cytotoxic T lymphocytes (CTL) are generated^{2,3}. Antigens coded by the central I region of the MHC are chiefly responsible for triggering prolifer-

Table 1 Collaborative effect of *in vitro* responses to antigens coded by the peripheral and central region of the H-2 complex

Responder cell	Stimulator cell (X irradiated)	H-2 incompatibility K,IA,IB,IC,D	³ H-thymidine uptake (c.p.m.)	% Specific lysis of targets CBA (k kkk k)	ATH (s sss d)
ATL (s kkk d)	ATL (s kkk d)	— — — — —	400 ±80	—1	—3
	ATH (s sss d)	— + + + —	22,000 ±3,600	6	24
	CBA (k kkk k)	+ — — — +	8,000 ±1,700	32	5
	ATH (s sss d)	— + + + —	27,000 ±3,100	87	35
	plus CBA (k kkk k)	plus			
	(CBA × ATH)F ₁ (s s s s d)	+ + + + +	29,000 ±2,600	92	34
	(k k k k k)				
	(k k k k k)				

The MLC system used to assay for proliferative responses (³H-thymidine uptake technique) is that described by Peck *et al.*²¹. Splenic responder cells (0.25×10^6 cells per well) were incubated with 0.25×10^6 X-irradiated (2,000 rad) splenic stimulator cells in MLC medium²¹ supplemented with 0.5% fresh mouse serum and 5×10^{-5} M 2-mercaptoethanol (ME) in flat bottom microtitre plates (Greiner, Nürtingen/Germany) over a period of 3.5 d. The cells were then pulsed with 1 μ Ci ³H-thymidine per well for 7 h and processed as described²². Six replicate cultures per group were formed. The data given represent the mean $\pm$ s.d. The culture system used for the development of CTL has been described elsewhere^{7,9}. Briefly, 3×10^6 responder cells were cultured together with 1.5×10^6 irradiated stimulator cells in Marbrook flasks containing MLC medium supplemented with 5% FCS and 5×10^{-5} M ME. After 5 d the cultured cells were titrated for cytotoxicity against LPS-induced blast lymphocytes⁹ in a ⁵¹Cr assay as described^{7,9}. The lysis values given (mean of three determinations) were obtained at a ratio of attacker cells to target cells of 30 to 1: the data are given without s.d., since in the experiments listed the s.d. of percentage specific lysis was less than [4] %. Background lysis during the 3-h assay was less than 18%. The letters within the brackets denote the H-2 genotype of the respective cells.

ation^{4,5}, whereas the target antigen of the CTL generated is either a H-2K or H-2D region or a I-A subregion gene product³⁻⁸. This dichotomy in the antigenic requirement of a MLC seems to be reflected at the level of the responding T lymphocytes. Two distinct subsets of T cells (T₁/T₂), which have been defined by both functional^{9,10} and physical¹¹ criteria and possibly on the basis of their Ly phenotype¹², seem to act synergistically during the *in vitro* generation of CTL⁹⁻¹³. Proliferating T₁ helper cells are thought to respond mainly to I-region gene products^{2,5,12} whereas the precursors of CTL (T₂ cells) are believed to react specifically to transplantation antigens coded by the H-2K, H-2D, or I-A region^{2,5,7}. The proliferating T₁ helper cell is believed to potentiate the development of CTL either by itself or through a secreted helper factor^{2,11,13,14}. A serious limitation of this concept is, however, that so far the experimental evidence available is derived from work performed *in vitro*. We therefore aimed at verifying T-T collaboration during *in vivo* responses to antigens of the MHC.

The mouse strains we used were chiefly strains ATL, ATH and CBA/J. CBA/J and ATL mice are identical for the H-2I region, whereas ATL and ATH mice share the same H-2K and H-2D regions, but differ at the I region¹⁵. The results presented in Table 1 were obtained *in vitro* and demonstrate the effect of allogeneic, I-region-coded determinants on the generation of CTL specific for antigens coded by the H-2K and H-2D region. Thus ATL responder T cells proliferate strongly in response to I-region-incompatible ATH stimulator cells. In this combination no CTL reactive against antigens of the H-2K or H-2D region of the H-2^k haplotype are generated (the CTL generated are reactive against antigens of the I-A^k subregion⁷). On the other hand, ATL responder cells respond with a moderate cell proliferation to H-2K and H-2D region-incompatible CBA stimulator cells, and anti-H-2K^k and anti-H-2D^k region-specific CTL are generated. In the presence of "third party" ATH stimulator cells, however, the generation of H-2K^k and H-2D^k region-specific CTL is strongly enhanced. These results basically confirm the work of Bach and associates^{3,13,16} and stress the collaborative effect of I-region-coded determinants on the *in vitro* generation of anti-H-2K and anti-H-2D region-specific CTL.

To find out whether during the induction of an *in vivo* allograft reaction a similar collaborative mechanism is operating, the following experimental protocol was applied:

a fixed number of distinct H-2 region-incompatible stimulator cells were injected into the hind footpad of ATL mice. After 3 d the draining lymph nodes were isolated, teased and single-cell suspensions were prepared. The cells were cultured (without adding stimulator cells) for 48–72 h more at 37 °C, a period required to allow full differentiation of the sensitised CTL precursor (A. St-P., in preparation). After this the lymph node cells were tested for the presence of CTL in a ⁵¹Cr-cytotoxicity assay. Results of a typical experiment are given in Table 2 and indicate *in vivo* an even more pronounced amplification effect of I-region incompatibility on the generation of H-2K and H-2D specific CTL compared with the *in vitro* data (Table 1). Thus the capacity of H-2K and H-2D region-incompatible stimulator cells to provoke a specific CTL response was greatly increased

Table 2 Collaborative effect of *in vivo* responses to antigens coded by the peripheral and central region of the H-2 complex

Recipient	Irradiated stimulator cells (10 ⁷)	H-2 incompatibility K,IA,IB,IC,D	% Specific lysis of targets CBA (k kkk k)	ATH (s sss d)
ATL (s kkk d)	ATL (s kkk d)	— — — — —	0	1
	ATH (s sss d)	— + + + —	4	19
	CBA (k kkk k)	+ — — — +	12	3
	ATH (s sss d)	— + + + —		
	plus CBA (k kkk k)	plus	76	27
	(CBA × ATH)F ₁ (s s s s d)	+ + + + +	69	24
	(k k k k k)			
	(k k k k k)			

ATL mice (recipients) were injected with either 10⁷ ATL, or ATH, or CBA, or F₁ (CBA × ATH), or a mixture of both 10⁷ CBA and 10⁷ ATH mouse derived irradiated splenic lymphocytes into the left hind footpad. After 3 d the draining lymph nodes (LN) were dissected, single-cell suspensions were prepared and the cells were cultured for 3 more days *in vitro*. In general about 4×10^6 – 6×10^6 cells were obtained per LN. Thereafter, the cells were titrated for cytotoxicity in a ⁵¹Cr assay⁷ against LPS-induced target cells⁷. The percentage specific lysis values given were obtained at a ratio of attacker cells to target cells of 30 to 1. Collaborative effects of I region-incompatible stimulator cells were obtained with as few as 2×10^6 ATH stimulator cells injected together with 10⁷ CBA stimulator cells (unpublished results). Background lysis of the target cells was less than 16%.

provided I region-incompatible stimulator cells were simultaneously injected into the hind footpad. In other words, the magnitude of the CTL response generated towards antigens coded by the peripheral K or D region of the H-2 complex was amplified by an I region-dependent stimulus presented separately on a "third party" cell.

From the experiments, we conclude that the collaborative effect of a T-cell response to I region-incompatible allogeneic stimulator cells on the generation of anti-H-2K and anti-H-2D-specific CTL seems to take place *in vivo* as effectively as *in vitro*. Since the target antigens of CTL are believed to be the transplantation antigens coded by the H-2K and H-2D region and I-A subregion of the H-2 complex^{5,7}, and since CTL are thought to be primarily responsible for allograft rejection *in vivo*³, the *in vivo* immunogenicity of grafted allogeneic cells seems to be strongly dependent on whether or not they express the phenotypical products coded by the I region of the H-2 complex, besides H-2 transplantation antigens. Because lymphoid cells express antigens coded by both the peripheral and central region of the H-2 complex, our results are compatible with the concept that passenger leukocytes¹⁷ may heighten the immunogenicity of transplanted allogeneic tissue grafts. They also offer an explanation of why anti-Ia antibodies eventually result in graft prolongation¹⁸, and are compatible with the finding that allogeneic thyroid transplants are rapidly rejected, provided the recipient's T lymphocytes are sensitised against I region coded cell surface antigens^{19,20}.

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Possible mechanism for the biological action of lithium

In spite of considerable early controversy it is now generally recognised that lithium has a favourable therapeutic effect on affective disorders and for some years lithium salts have been used with some degree of success in medical practice^{1,2}. Curiously, in spite of the extreme simplicity of lithium as a drug, there is little understanding of its mode of action. In this article we outline a possible explanation.

Lithium as a drug is given in the form of a simple salt and

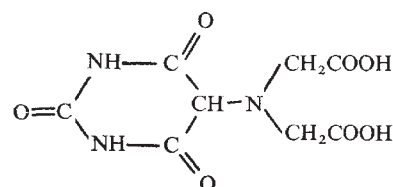


Fig. 1 Uramildiacetic acid

the active agent is undoubtedly the lithium cation. The dose level, around 1 mM, in the body, is extremely high and the most likely action of lithium is that it challenges one of the common biological cations Na^+ , K^+ , Mg^{2+} and Ca^{2+} . This explanation meets, however, with an immediate difficulty in that it is very hard to find an example of an organic ligand which can bind lithium with a stability constant as high as 10^8 and yet does not bind Mg^{2+} and Ca^{2+} with much higher affinity (Table 1). In other words, Table 1 seems to show that there is no ligand which lithium could bind in the presence of these cations. In this note we wish to show that the use of Table 1 in this fashion is completely misleading as to the possible action of lithium within the context of biological media, which amounts to saying that chemical affinities measured by absolute values of stoichiometric stability constants do not give the true tendency for preferential binding of a certain metal ion if other potential complexing agents are also present and that the free metal ion content of the compartment under discussion must be known. A better approach is to use 'conditional' stability constants, which are constants valid for the medium in which the reaction is taking place. The discussion then has much in common with that used in analytical chemistry where less stable metal complexes are preferentially formed by a metal M_1 with a ligand L_2 by 'removal' of other metals M_2 by 'sequestering' or 'masking' agents L_1 which bind M_2 differentially more strongly, that is, under the conditions of the experiment $K_{\text{M}_2\text{L}_1} \gg K_{\text{M}_1\text{L}_1}$ such that $K'_{\text{M}_1\text{L}_2} > K'_{\text{M}_2\text{L}_2}$.

We start the analysis of the binding of the five cations Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} to different ligands by using as a convenient measure of the uptake tendency of a certain ion by a certain ligand the product $K'_{\text{ML}} [\text{M}]$, where K'_{ML} is the

Fig. 2 Conditional constants of the magnesium and lithium complexes of uramildiacetic acid ($\log K'_{\text{ML}}$, at pH = 7) as a function of the concentration of free ATP^{4-} .

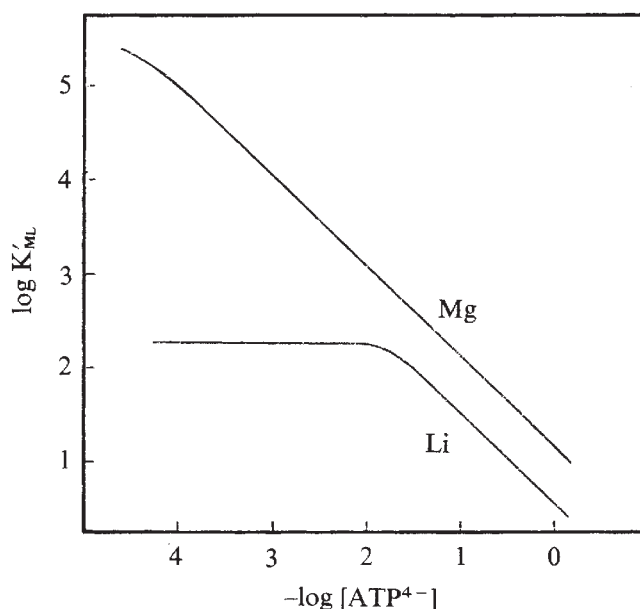


Table 1 Stability constants* (log K) of complexes of alkali and alkaline earth complexes in aqueous solution⁷

Ligand	Li ⁺	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	T(°C) μ†
OH ⁻	0.3	-0.5	—	2.5	1.3	25/0
HPO ₄ ²⁺	0.5	0.1	—	2.5	(1.5)	25/0
P ₂ O ₇ ²⁻	2.39	1.0	0.8	5.41	4.95	25/1
P ₃ O ₁₀ ⁵⁻	2.8	1.7	1.3	5.83	5.44	25/1
ADP ³⁻	—	0.65	0.67	3.23	2.81	25/0.2
ATP ⁴⁻	1.7	1.2	0.9	4.43	3.92	25/0.3 or 0.1
Uramildiacetate	4.9	2.72	1.23	8.12	8.31	20/0.1
<i>o</i> -Hydroxyphenyliminodiacetate	2.2	1.0	—	6.86	6.27	20/0.1
<i>o</i> -Carboxyphenyliminodiacetate	2.05	0.89	—	3.91	5.06	20/0.1
Cyclohexyliminodiacetate	1.74	0.90	—	3.46	3.34	20/0.1
2-Hydroxycyclohexyliminodiacetate	2.19	0.75	—	4.27	5.19	20/0.1
<i>o</i> -Sulphophenyliminodiacetate	2.26	0.98	—	2.68	4.57	20/0.1
Iminodiacetate	0.96	0.36	—	2.94	2.59	20/0.1
Methyliminodiacetate	1.20	0.61	—	3.44	3.75	20/0.1
Nitrilotiracetate	2.51	1.32	0.6	5.41	6.41	20/0.1
EDTA ⁴⁻	2.79	1.66	0.96	8.69	10.70	20/0.1
MethylEDTA ⁴⁻	3.43	2.24	—	10.29	11.47	30/0.1
EGTA ⁴⁻	1.2	1.4	—	5.41	11.0	25/1.5 or 0.1
DCTA ⁴⁻ (ref. 4)	4.13	2.70	—	10.35	11.20	30/0.1
Cryptate 112 (ref. 5)	4.30	2.80	<2	—	2.80	25/0.1

†μ, Ionic strength.

*Source: *Stability Constants* (Chem. Soc. Sp. Publs 17 and 25, London 1964, 1971).

'conditional' stability constant of the complex formed between that ion and ligand concerned and [M] is the concentration of metal ion in solution in the compartment³.

In the case of prolonged lithium therapy, the concentrations of the common alkali- and alkaline-earth metals in the cytoplasm of cells will be close to those given in Table 2. In the cells there are not many good ligands of comparable concentration but, strikingly, adenosine triphosphate (ATP) is often present in a very comparable concentration range. In some vesicles, such as the adrenal chromaffin granule, the ATP concentrations may reach even higher values; typical results are Ca²⁺ — 14 mM, Mg²⁺ — 6 mM, K⁺ = 20 mM ATP — 100 mM (ref. 4 and A. Daniels and R. J. P. W., unpublished). Leaving such special regions of the cell aside for the moment, we see that inside the cytoplasm of cells generally lithium will be selected preferentially to sodium and potassium if log K'_{Li} is greater than log K'_{Na} and log K'_K by a factor of 2. Several ligands meet this requirement. Lithium will be selected preferentially to calcium by any ligand which has a conditional stability constant which is not more than 3 log units smaller for the lithium complex and even up to a difference of 5 log units there will be appreciable competition of lithium for calcium sites. This is easily achieved by many ligands which have log K'_{Li} of about 3 (that is conditional constant at pH = 7). Thus the calcium ion in cells will not prevent lithium binding. Magnesium poses a greater problem for it is present in equal or greater concentration than lithium and we must now examine competition between ligands for magnesium and lithium.

Let us take the situation in the cytoplasm when Mg²⁺ (total) < ATP (total) and examine the effect of this background of relatively high ATP concentration upon the conditional stability constant of lithium and magnesium complexes with some chosen ligands (Table 1).

One of the most stable lithium complexes known is that of

uramildiacetic acid ligand (Fig. 1)—which has been studied in detail by one of us^{6,7}.

The values given in Table 2 were used to calculate 'conditional' stability constants for the magnesium and the lithium complexes of uramildiacetic acid in the presence of 10^{-2.5} M free ATP. The results are presented graphically in Fig. 2 and it can be seen that for the conditions prevalent inside cells, as described above, although the magnesium complex remains the most stable of the two, lithium is likely to interfere and compete with magnesium to some 20–30% for its binding sites.

It is perhaps worthwhile to point out that the choice of uramildiacetic acid is not arbitrary, as there are obvious analogies between uramildiacetic acid and some compounds from which it actually derives, for example, barbituric acid and uric acid, curiously enough associated with lithium therapy from the very beginning. Hence Lipowitz and Garrod⁸ refer to the combination of lithium with uric acid promoting the dissolution of urate deposits in cartilages and, more recently, Margulies⁹ used lithium in combination with barbiturates obtaining favour-

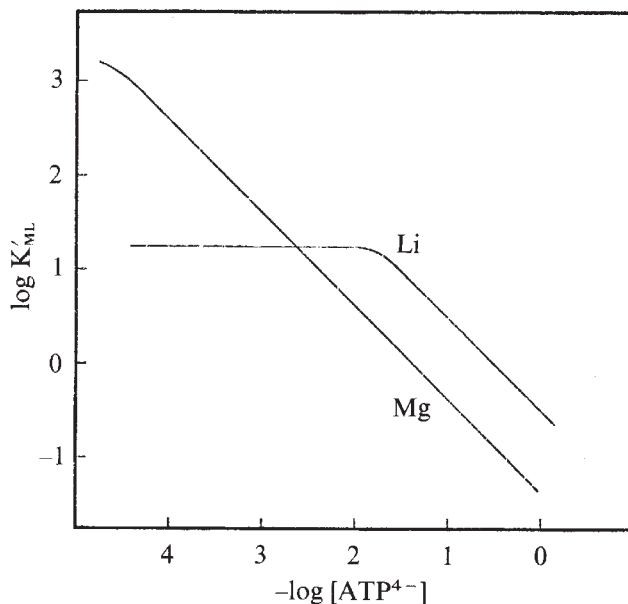
Fig. 3 Conditional constants of the magnesium and lithium complexes of *o*-carboxyphenyliminodiacetic acid (log K'_{ML}) at pH = 7 as a function of the concentration of free ATP⁴⁻.

Table 2 Concentration of common alkali earth metals and of ATP in cytoplasm of cells

Species	Concentration (M)
Li ⁺	10 ⁻³
Na ⁺	10 ⁻²
K ⁺	10 ⁻¹
Mg ²⁺	10 ⁻³ —10 ^{-2.5}
Ca ²⁺	10 ⁻⁷
ATP	10 ^{-3.0} —10 ^{-2.5}

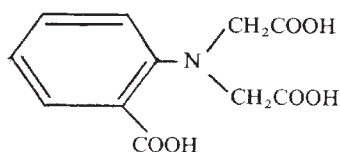


Fig. 4 Ortho-carboxyphenyliminodiacetic acid.

able results in the treatment of manic states. Protective effects on urea toxicity in urine from manic-depressive patients injected to guinea pigs was also reported by Case¹⁰.

Nevertheless, uramildiacetic acid as such does not occur in biological systems and the conclusion to be derived from the result expressed in Fig. 1 is that chelating sites of liganding atoms (O^- , N , O^- , O^-) are likely to exhibit increased affinity for lithium and, in the presence of alkaline-earth metal sequestering agents, this metal may compete to a greater or lesser extent with Mg^{2+} and Ca^{2+} for the above mentioned sites.

As a matter of fact it is not necessary that the ligands have a particularly high affinity for lithium, if it is high enough to give appreciable complex formation. What is more important is that the gap of stability between the lithium and the magnesium complexes is made as small as possible while keeping the values of the constants above 2 log units. In Fig. 3 we present the values obtained for the lithium and magnesium complexes of *o*-carboxyphenyliminodiacetate (Fig. 4), which has a coordinating site (O^- , N , O^- , O^-) similar to that of uramildiacetic acid, although it is not such a powerful complexing agent¹¹. It can be seen that for concentrations of free ATP of the order of $10^{-2.5}$ M the lithium and magnesium complexes are of equal 'effective' stability and for higher concentrations of ATP the lithium complex actually becomes stronger than the magnesium complex; lithium will then be taken up preferentially by this (O^- , N , O^- , O^-) ligand, although $\log K'_{Li}$ at $pH = 7$ is not sufficiently high to ensure the predominance of the complex species.

Other similar examples can be given and although the ligands referred to above do not exist *in vivo*, it is likely that analogous or even more adequate coordinating sites may have developed. The variety of sites provided *in vivo* for the uptake of the various elements and the characteristics of those for calcium and magnesium make this idea more than a simple guess or prediction, see, for example, concanavalin¹².

If we consider proteins as ligands, it is obvious that sites of the kind (O^- , N , O^- , O^-) could have evolved, that they would bind Mg^{2+} , but that this binding would be considerably affected by the concentration of ATP and, finally, that in this complicated situation there could be a further weakening of binding through the influence of added lithium. Now, while a situation in which $(Mg^{2+}) = 10^{-3}$ M and $(ATP) = 10^{-2.5}$ M might be thought to be somewhat artificial in the cytoplasm of cells in general, just such a situation arises in the internal fluids of the adrenal chromaffin granule and it could occur in many other vesicles, for example, in the transmitter vesicles of nerve cells. Thus it could well be that Li^+ is indeed challenging Mg^{2+} and so affecting metabolic rates as diverse as the action of glycolysis to the rates of many ATP-driven transfer proteins.

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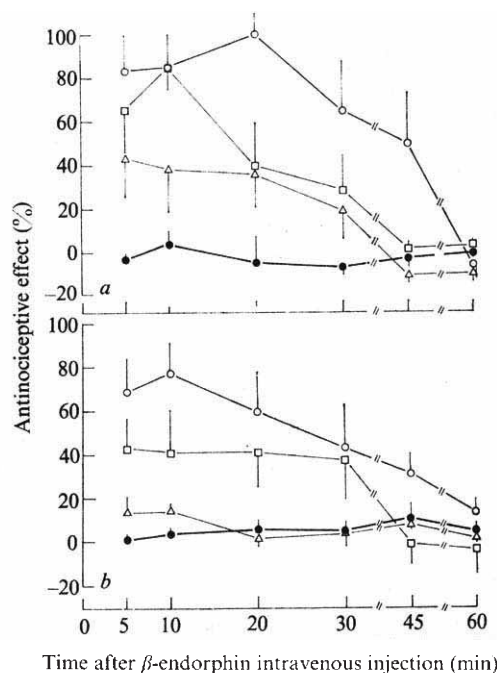
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β -Endorphin as a potent analgesic by intravenous injection

AN untriatkontapeptide¹ with significant opiate activity from camel pituitary glands was shown to have an amino acid sequence identical to the carboxyl-terminal 31 amino acids of β -lipotropins^{2,3}. The peptide, designated as β -endorphin, has recently been synthesised⁴, and when administered directly into the brain, it was found⁵ to be considerably more potent than morphine in molar basis, and its actions were blocked by naloxone. Other peptides related to β -lipotropins have also been shown to possess *in vitro* opiate-like activity⁶⁻⁹. We report here the analgesic effects of β -endorphin when it is injected intravenously.

Male ICR mice weighing 25-30 g (Simonson Laboratories) were used in all the experiments. β -Endorphin was synthesised as described previously⁴. Naloxone HCl was a gift from Endo Laboratories and morphine sulphate was purchased from Mallinckrodt Chemical Works. The antinociceptive or analgesic

Fig. 1 Antinociceptive effects following the intravenous injection of β -endorphin in (a) the tail-flick test and (b) hotplate test, and its blockage by naloxone. Mice, 6-7 per group, were injected intravenously with 8.2 (Δ), 14.5 ($\square$), and 20.1 ($\circ$) mg kg^{-1} of β -endorphin at 0 time. Naloxone HCl (1 mg kg^{-1}) ($\bullet$) was injected subcutaneously 5 min before the intravenous injection of β -endorphin. 'Percentage' antinociceptive effect was calculated¹⁰ as $[(T_1 - T_0)/(T_2 - T_0)] \times 100$, where a control latency (T_0) was obtained from the mean of two latencies determined before drug injection: test latencies (T_1) were determined at various times after injection for each animal; the cutoff times (T_2) for the tail-flick test and hotplate test were 7 and 60 s, respectively. The vertical bars indicate the s.e.m.



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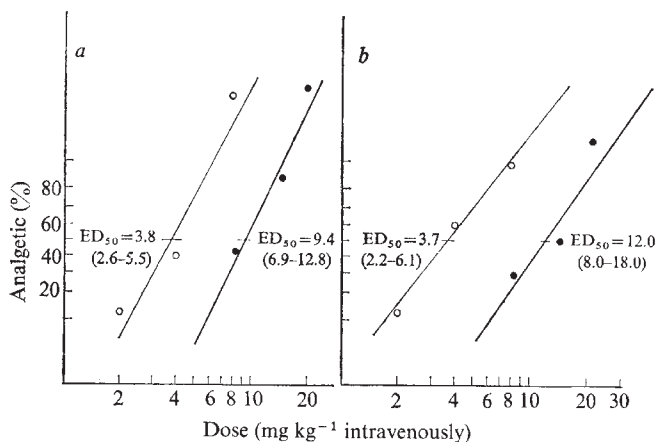


Fig. 2 Comparison of antinociceptive effects of β -endorphin (●) and morphine (○) in tail-flick test (a) and hotplate test (b). After control latency was obtained before the drug injection, groups of mice, 6–10 in each, were injected intravenously with various doses of β -endorphin or morphine sulphate and the antinociceptive effect was determined 10 min after drug injection. The values in the figure are median antinociceptive dose (ED_{50}) in $mg\ kg^{-1}$ and the 95% confidence limits are shown in the parentheses. When the ED_{50} values are expressed on the molar basis, the ED_{50} of β -endorphin is $2.7\ \mu mol\ kg^{-1}$ and of morphine $11.4\ \mu mol\ kg^{-1}$ in the tail-flick test. The ED_{50} of β -endorphin is $3.5\ \mu mol\ kg^{-1}$ and of morphine $11.1\ \mu mol\ kg^{-1}$ in the hotplate test.

properties of β -endorphin and morphine were assessed by both the hotplate¹⁰ and the tail-flick¹¹ methods. β -Endorphin and morphine sulphate were injected intravenously into the tail vein in a volume of 0.01 ml per g body weight. The extent of analgesia or antinociceptive response was expressed as 'percentage antinociceptive effect' (see ref. 12).

With a twofold increase in latency of reaction times as a quantal index of inhibition, the median antinociceptive dose (ED_{50}) and 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon¹³.

β -Endorphin administered at intravenous doses of 8.2, 14.5 and $20.1\ mg\ kg^{-1}$ produced a dose-related inhibition of both the tail-flick and the hotplate response of mice to nociceptive stimuli (Fig. 1). These effects lasted 30–60 min, depending on the dose used, and seemed to be shorter than the duration of β -endorphin when applied centrally⁵. Pretreatment of mice with naloxone HCl ($1\ mg\ kg^{-1}$, subcutaneously) 5 min before the injection of $20.1\ mg\ kg^{-1}$ of β -endorphin completely eliminated the inhibitory effect of β -endorphin on the tail-flick and hotplate responses. Thus, in two tests for antinociceptive activity, β -endorphin injected intravenously was as effective as when it was administered directly into the brain.

The percentage of mice which were analgetic after intravenous injection of various doses of β -endorphin and morphine, and their ED_{50} values with 95% confidence limits are given in Fig. 2. The ED_{50} values expressed in $mg\ kg^{-1}$ of β -endorphin were about 2–3 times higher than that of morphine. When the potency was compared on a molar basis, β -endorphin was ~3–4 times more potent than morphine. We have previously shown that β -endorphin was ~18–33 times more potent than morphine when applied centrally⁵. Since the molecular weight of β -endorphin is ~10 times higher than morphine, it is likely that the lower potency ratio of β -endorphin to morphine when they are applied peripherally is due to the relatively poorer penetration of β -endorphin into the brain. In addition, a different rate of degradation after peripheral administration is a possible factor.

We have shown that β -endorphin can produce analgesia when administered intravenously and the analgesic effect can be eliminated by pretreatment with naloxone. This work was

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Comparative study on analgesic effect of Met⁵-enkephalin and related lipotropin fragments

HUGHES *et al.*¹ have reported the isolation and structure of two pentapeptides from porcine brain with opiate agonist activity in isolated systems. The structure of one of these peptides, Met⁵-enkephalin, is identical with the sequence of pituitary β -lipotropin (β -LPH) between residues 61–65 (refs 2–4). To prove the biological correlation of brain enkephalin and pituitary β -LPH, a series of lipotropin fragments, LPH-(61–69)-⁵, LPH-(61–76)-⁶ and LPH-(61–91)-peptides^{7–10}, have been isolated and shown to have opiate agonist activity *in vitro*. Only few and controversial data have been available so far on the analgesic effect *in vivo* of the above or similar lipotropin fragments. Enkephalins have recently been reported to induce analgesia *in vivo*^{11,12}. Our preliminary data^{3,13}, however, seemed to contradict these observations, rather suggesting that some larger fragment(s) of β -LPH may have analgesic properties. We have therefore compared the analgesic effects of Met⁵-enkephalin and some lipotropin fragments containing the complete structure of Met⁵-enkephalin at their NH₂-terminus. The results show that the *in vivo* effect is a function of the length of the peptide chain, Met⁵-enkephalin being the least and LPH-(61–91)-peptide the most potent. During the preparation of this paper we have become aware of the recent observation of Bradbury and coworkers^{10,14} on a strong analgesic activity of LPH-(61–91)-peptide.

Met⁵-enkephalin was synthesised as described previously⁵. LPH-(61–79)-peptide was obtained by plasmin cleavage of porcine β -LPH^{15–17}. LPH-(61–91)-peptide was isolated from a crude adrenocorticotrophic hormone preparation by high voltage paper electrophoresis at pH 6.5 (ref. 9). The *in vitro* opiate agonist activity of the fragments was determined on longitudinal muscle strip of guinea pig ileum¹⁸. For the *in vivo* assay the classical tail-flick technique was used according to D'Amour and Smith¹⁹. The tail of male CFY rats was exposed to a radiant heat and the latency of tail withdrawal measured automatically with the accuracy of 0.1 s. The cutoff time was 15 s. All the substances studied were dissolved in saline and administered intra-

cerebroventricularly as described by Chermat and Simon²⁰, in a volume of 20 μ l.

In the *in vitro* assay the potency order was found to be as follows: LPH-(61-91)-peptide=LPH-(61-65)-peptide>LPH-(61-69)-peptide=LPH-(61-79)-peptide (Table 1). The 70-90% inhibitory effect of these fragments could be reversed by 100 nM naloxone.

Using the schedule described by Belluzzi *et al.*¹¹ for measurement the analgesic effect of peptides—that is, measuring the latency time every 2 min for 10 min—the prolongation of the reaction time was moderate for all the materials studied, including morphine. In addition, the effect of enkephalin was short lasting, subsiding 6-8 min after application (Fig. 1a).

This type of *in vivo* effect significantly differs from that caused by either morphine or larger lipotropin fragments since the maximal effect of the latter compounds did not develop within such a short period of time. As Fig. 1b shows, using another time schedule—that is, controlling the tail withdrawal reaction for 2 h—a marked analgesic activity was obtained for morphine, LPH-(61-91)- and LPH-(61-79)-peptides, but only a moderate and transitory prolongation of the tail-flick reaction could be achieved by Met⁵-enkephalin. The three stronger analgesic compounds were characterised by a slow onset of action which reached a plateau at 15-30 min after administration and lasted for 60-120 min. Their activity could be antagonised by subcutaneously administered naloxone at a dose of 2 mg kg⁻¹, 30 min before intracerebroventricular treatment.

The *in vitro* and *in vivo* opiate agonist activities of Met⁵-

Fig. 1 Time-response curves for the analgesic activities of morphine and β -LPH fragments administered intracerebroventricularly to rats. Abscissa: time elapsed before (—) and after intracerebroventricular administration of materials (at time 0). Latency time was measured either every 2 min (a) or using longer intervals between the measurements as indicated on the scale (b). $\square$, Morphine 33 nmol per animal ($n = 20$); $\circ$, LPH-(61-91)-peptide 0.8 nmol per animal ($n = 6$); $\bullet$, LPH-(61-65)-peptide 670 nmol per animal ($n = 23$); $\times$, saline 20 μ l per animal ($n = 21$) for a; and $\square$, morphine 33 nmol per animal ($n = 19$); $\circ$, LPH-(61-91)-peptide 0.8 nmol per animal ($n = 6$); $\blacktriangle$, LPH-(61-79)-peptide 40 nmol per animal ($n = 5$); $\bullet$, LPH-(61-65)-peptide 670 nmol per animal ($n = 10$); $\times$, saline 20 μ l per animal ($n = 16$) for b, respectively.

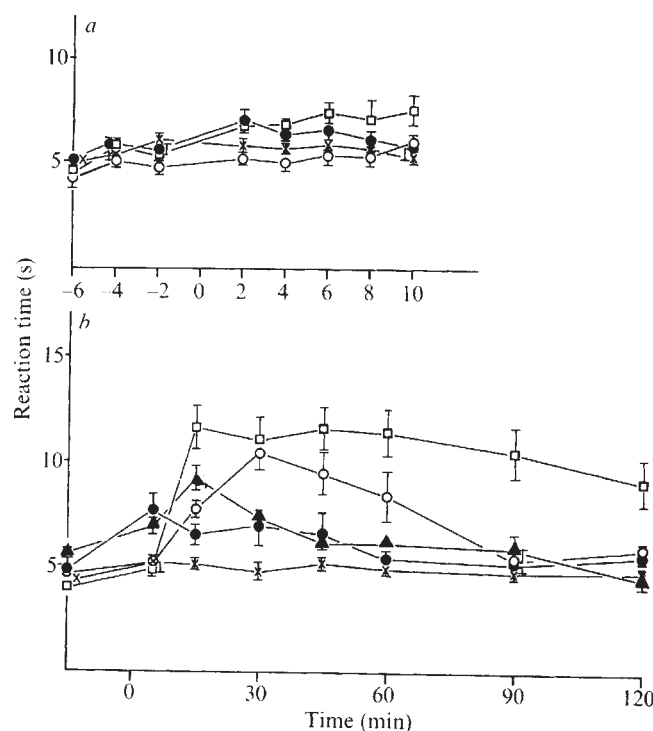


Table 1 Opiate agonist potencies of porcine β -LPH fragments in longitudinal muscle strip of guinea pig ileum

Fragment	Relative agonist potencies*	Statistical evaluation†
LPH-(61-65)-peptide	1 ($n = 4$)	—
LPH-(61-69)-peptide	0.51 ± 0.12 ($n = 4$)‡	$P < 0.05$
LPH-(61-79)-peptide§	0.42 ± 0.06 ($n = 7$)	$P < 0.005$
LPH-(61-91)-peptide§	1.37 ± 0.16 ($n = 4$)	NS

*Potency ratios were calculated from ID₅₀ values. The dose-response curves for the peptides were parallel.

†The statistical comparison was based on the ID₅₀ values, using the unpaired *t* test.

‡Mean $\pm$ s.e.m. values are listed; the numbers of experiments are in parentheses.

§Guillemin *et al.*⁶ and Li and Chung⁷ have proposed to designate LPH-(61-76)-, LPH-(61-77)- and LPH-(61-91)-peptides as α , γ and β endorphins, respectively. Considering that LPH-(61-79)-peptide also occurs in the pituitary gland (unpublished results), and also that it is closely similar to γ endorphin, we propose to designate it as δ endorphin.

enkephalin (Table 1, Fig. 1) indicate that the molecule contains all the essential residues for the interaction with the opiate receptors. The relative inefficiency and short duration of the *in vivo* effect induced by Met⁵-enkephalin may be due to its rapid degradation by brain enzymes, as also suggested by Belluzzi *et al.*¹¹. The more pronounced and prolonged analgesic effect obtained with LPH-(61-79)-peptide strongly supports this view (Fig. 1b). LPH-(61-79)-peptide is less potent than Met⁵-enkephalin on longitudinal muscle strip of guinea pig ileum. Consequently the function of the COOH-terminal portion (residues 66-79) of the molecule in increasing the *in vivo* analgesic effect of the peptide may be the protection of the active site (residues 61-65) against the proteolytic enzymes of the brain. A similar protecting role has been attributed to the COOH-terminal portion of adrenocorticotrophic hormone (residues 25-39)^{21,22}. Among the fragments studied, LPH-(61-91)-peptide showed the highest opiate agonist activity *in vitro* (Table 1). This finding, together with the extremely high *in vivo* analgesic effect of this fragment (at least 50 times higher than that of morphine), suggests that additional receptor binding site(s) may be present within residues 80-91 of the molecule.

Our *in vivo* results and also their explanation seem to be in agreement with the recent data of Bradbury *et al.*¹⁰ on the *in vitro* receptor-binding properties of similar fragments.

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Selective activation of the mesocortical DA system by stress

It is now well established that an important system of dopaminergic (DA) neurones innervates various parts of the cerebral cortex in the rat and other species¹⁻³. In contrast to noradrenergic (NA) terminals which are widely distributed in this structure, the DA terminals are mainly confined to deep layers, particularly in the frontal, the cingulate and the entorhinal areas^{4,5}. The results of lesion studies demonstrated that the terminal endings in the frontal cortex originate from the A10 group of DA cell bodies localised in the mesencephalon⁶⁻⁸. This group also contains the cell bodies of the classical mesolimbic DA system projecting to the tuberculum olfactorium, the nucleus accumbens, the nucleus of the stria terminalis, and the amygdala⁹. The DA terminals found in the cingulate and entorhinal areas of cortex may originate mainly from the A9 group of DA neurones⁶⁻⁸. This group gives rise to the well known nigrostriatal DA system which is implicated in extrapyramidal processes. Its degeneration is in part responsible for some of the symptoms seen in Parkinsonian patients. Little is yet known about the functions of the mesocortical and mesolimbic DA pathways. Electrocoagulation or 6-hydroxydopamine (6-OHDA)-induced lesions of the ventral tegmental area, containing the A10 group, produce a syndrome characterised by "locomotor hyperactivity, serious impairment in tests requiring inhibition of a previously learned response, facilitation of approach learning and of active avoidance and hypoemotivity"^{10,11}. Various workers have suggested that the antipsychotic effects of neuroleptics are in part related to the blockade of postsynaptic DA receptors localised in areas innervated by the mesolimbic and mesocortical DA systems^{12,13}. It seems important to establish whether neurones of these two DA systems correspond to an homogeneous population of cells with similar functional characteristics. We have therefore explored this problem in the rat by examining the reactivity of the mesocortical and mesolimbic DA pathways as well as that of nigrostriatal DA system to stress induced by electric foot shocks. Our results suggest that the mesocortical DA system is selectively activated by this stress.

Groups of male Charles River rats of the Sprague-Dawley strain, kept in a controlled environment (24 °C, 60% humidity, alternate cycles of 12 h light—0700–1900—and 12 h darkness 1900–0700) and receiving food and water *ad libitum* were injected at 1400 with α -methylparatyrosine (200 mg kg⁻¹ intraperitoneally), an inhibitor of catecholamine (CA) synthesis. Some of them then received electric foot shocks 10 min later, using a procedure previously described¹⁴. Others were used as controls. All animals were killed 30 min after administration of the synthesis inhibitor. Changes in CA concentrations were estimated in the medial part of the frontal cortex, the nucleus accumbens and the tuberculum olfactorium, and in the striatum. Since the two first areas are relatively poor in DA or consist of only small amounts of tissue, they were dissected precisely from frozen frontal slices (500 μ m thick) using the microdisk technique originally described by Palkovits¹⁵. Particular care was taken to punch out areas rich in DA fibres

and 3H-DA uptake sites in the medial part of the frontal cortex⁸ between planes 9,800 and 12,800 μ m; the beginning of the striatum at 9,800 μ m according to the atlas of König and Klippel¹⁶ was used as a landmark. The nucleus accumbens was also punched out with a tube sharpened at the extremity (0.8 mm diameter) from two slices delimited by the 9,800- and 8,800- μ m planes. Pooled microdisks of tissues corresponding to each area were sonicated in 80 μ l of 0.1 N perchloric acid and 0.01 N thioglycolic acid solution. After centrifugation, DA and NA were isolated from the supernatant on alumina microcolumns¹⁷, and measured using the sensitive radioenzymatic microassay of Gauchy *et al.*¹⁷. Pellets were dissolved in 0.1 N NaOH for protein estimations¹⁸. Since the tuberculum olfactorium and the striatum contain large amounts of DA, they were dissected at 4 °C and the amine was isolated by ion-exchange chromatography on Amberlite CG50 and estimated spectrofluorimetrically¹⁴. To compare the results obtained by the two procedures, in one experiment, DA was estimated radioenzymatically in two microdisks punched out in the mediolateral part of the striatum from two slices (500 μ m thick) delimited by the 8,500- and 7,500- μ m planes.

Changes in the rate of disappearance of CA after synthesis inhibition provide reliable indications of modifications in the rate of amine utilisation. The electric foot shock stress did not affect the rate of DA utilisation either in the whole striatum or in its mediolateral part, since DA levels were identical in control and stressed animals (Fig. 1.) Similar observations were made in the tuberculum olfactorium. In the nucleus accumbens, however, which is another structure innervated by the mesolimbic DA system, the utilisation of DA was accelerated as indicated by the 25% decrease in DA levels in tissues from stressed animals when compared with those of respective controls (Fig. 1). The stress effect was much more striking in the

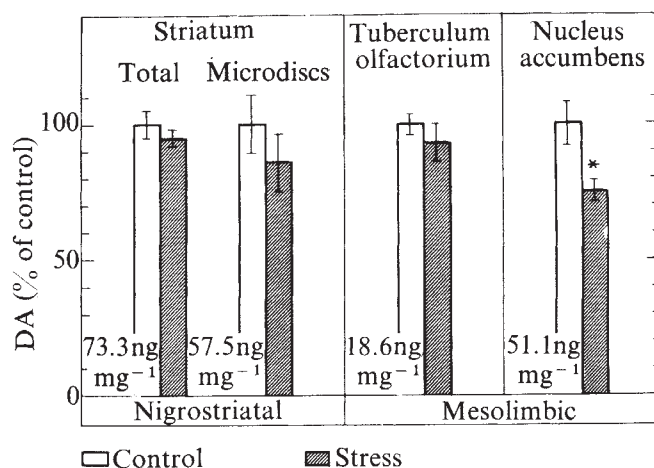


Fig. 1 Effect of 20-min electric foot shock stress on the utilisation of DA in structures innervated by the nigrostriatal or the mesolimbic DA pathways. DA was estimated spectrofluorimetrically (total striatum, tuberculum olfactorium) or radioenzymatically (striatal microdisks, nucleus accumbens) 30 min after the injection of α -methylparatyrosine (200 mg kg⁻¹, intraperitoneally) in tissues of control and stressed rats. The stress was applied 10 min after the injection of inhibitor. In the radioenzymatic assay, α -methyl dopamine is measured concomitantly with DA. However, α -methyl dopamine formed from α -methylparatyrosine represented less than 10% of DA levels 30 min after the inhibitor injection (A. Chéramy, personal communication). Numbers in bars represent absolute mean values of DA (ng per mg protein). Data expressed as % of respective control values are the mean $\pm$ s.e.m. of results obtained with groups of 6 rats. $P < 0.02$ when compared with respective controls. DA levels in animals not treated with α -methylparatyrosine were for total striatum, striatal microdisks tuberculum olfactorium and nucleus accumbens: 90.0 ± 6.0 ng mg⁻¹, 75.0 ± 5.0 ng mg⁻¹, 22.3 ± 2.1 ng mg⁻¹ and 79.1 ± 7.0 ng mg⁻¹ respectively.

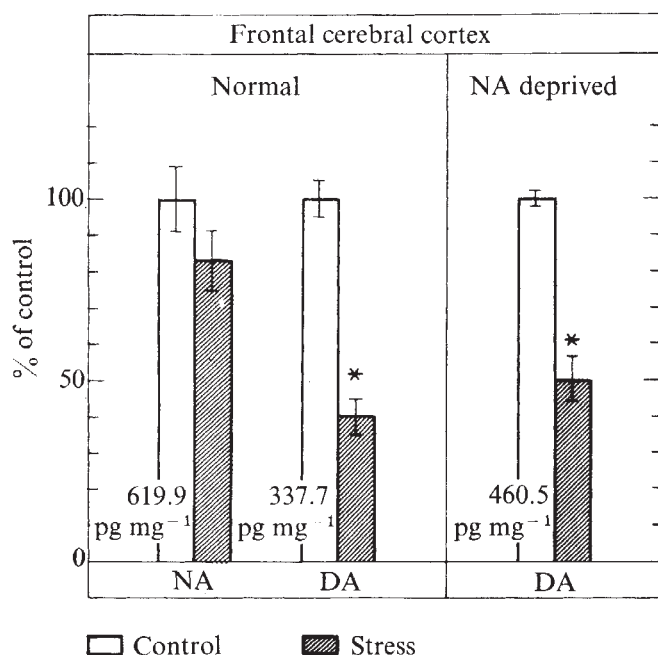


Fig. 2 Effects of a 20-min electric foot shock stress on the utilisation of DA in the frontal cerebral cortex of normal rats and of animals deprived of cortical NA innervation (NA deprived). Selective degeneration of ascending NA pathways was induced by local 6-OHDA injections 12 weeks before the stress experiment. All rats were submitted to the 20-min stress situation 10 min after the injection of α -methylparatyrosine (200 mg kg⁻¹ intraperitoneally). DA and NA levels were estimated radioenzymatically in the frontal cerebral cortex 30 min after the inhibitor injection (numbers in bars represent the absolute mean values of NA and DA expressed as pg per mg protein). Data expressed as % of respective control values are the mean $\pm$ s.e.m. of results obtained with groups of six rats. In rats deprived of noradrenergic innervation, cortical NA levels were less than 10% those of controls. $P < 0.001$. Cortical DA and NA levels in normal rats not treated with α -methylparatyrosine were 720 ± 30 pg mg⁻¹ and 950 ± 100 pg mg⁻¹.

frontal cerebral cortex, in which a 60% reduction in DA levels was observed in animals submitted to electric foot shocks (Fig. 2). The short period of stress did not affect the rate of NA utilisation in this structure.

The effect of stress on the mesocortical DA neurones was further studied in rats deprived of a NA innervation to the cerebral cortex. For this complementary experiment, the ascending NA systems were destroyed by a local micro-injection of 6-OHDA (2 μ g in 1 μ l of isotonic saline solution) made laterally to the pedunculus cerebellaris superior¹, 12 weeks before the stress experiment. Two groups of rats were injected as before with α -methylparatyrosine and one of them was exposed to the stress situation. As indicated in Fig. 2, the rate of DA utilisation was accelerated in the stressed rats in a similar manner to that observed in unlesioned animals. As expected, NA could no longer be detected in the frontal cerebral cortex of the lesioned rats. It thus seemed that the stress-induced activation of the mesocortical DA neurones was unrelated to the ascending NA systems.

In previous studies¹⁴, we reported that an electric foot shock stress of long duration accelerated both the utilisation and the turnover of NA in various structures of the brain; the effect being particularly striking in the brainstem. We assumed that these changes in NA metabolism were related to modifications in neuronal activity. The stress of long duration (180 min) did not influence DA utilisation in striatal DA terminals¹⁴. It is thus not surprising that the 20-min stress used in the present study did not modify the activity of the nigrostriatal DA neurones. In fact, other

stress situations such as exposure to noise or photic stimuli, intense muscular exercises, or exposure to cold, were found to have no effects on DA metabolism in the striatum. In the present study, it was interesting to note that two structures belonging to the mesolimbic DA system did not react similarly to the 20-min stress situation. Indeed DA utilisation was accelerated significantly in the nucleus accumbens but not in the tuberculum olfactorium. This demonstrates that the various DA neurones of the mesolimbic system do not behave similarly. We have already indicated that the DA terminals localised in the medial part of the frontal cortex also originate from the A10 group of DA cell bodies. The dramatic effect of electric foot shocks on the activity of these mesocortical neurones further revealed the heterogeneity of the A10 group. The very pronounced and selective acceleration of DA utilisation in these neurones indicates that the mesolimbic and mesocortical DA systems can be distinguished not only on anatomical grounds but also from a functional point of view. From these results, it is doubtful that the DA terminals present in the various limbic structures and in the frontal cortex originate from the same DA cell bodies. Recent results on the effects of chronic neuroleptic treatments on the synthesis of DA in structures innervated by the mesolimbic and mesocortical DA pathways support the above statements¹⁹. Indeed, the time curves of the effect of neuroleptics on DA synthesis in the frontal cerebral cortex and in the tuberculum olfactorium+nucleus accumbens were different.

In an earlier study, Bliss and Ailion²⁰ observed an increase in the content of homovanillic acid (a major DA metabolite) in the whole brains of rats or mice submitted to severely stressful experiments. They suggested that DA neurones could be involved in the cerebral circuit of emotionality. In this context, the high and selective sensitivity of the mesocortical DA neurones to electric foot shock stress revealed in our study is of particular interest. This DA system originates from the "limbic midbrain area" and projects to the frontal cerebral cortex, connected directly and indirectly to the limbic structures. It may be that this mesocortical DA pathway has a critical role in the regulation of emotional states.

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Lesion of striatal neurones with kainic acid provides a model for Huntington's chorea

THE symptoms of Huntington's chorea, an hereditary movement disorder, result from degeneration of neurones primarily in the basal ganglia¹. Several neurochemical abnormalities have been identified in the brains of patients dying with this disorder^{2–5}, but no animal system with similar neuropathological changes has been described. We now report that the injection of kainic acid into the rat striatum causes neuronal degeneration, neurochemical alterations and behavioural responses resembling Huntington's chorea. This procedure could provide an animal model for the study of the disease.

Systemic administration of monosodium glutamate to immature mice causes degeneration of certain neurones of retina⁶ and brain⁷. In brain, neurones with cell bodies in the arcuate nucleus, which selectively accumulates glutamate⁸, degenerate whereas axons passing through or terminating in the region are unaffected⁹. The neurotoxic effects of glutamate may be due to its ability to depolarise neurones, for the neurotoxicity of several dicarboxylic and sulphur-containing amino acids structurally related to glutamate correlates with their neuroexcitatory action¹⁰. In addition, neuronal sensitivity to depolarisation by glutamate is limited to dendrites and soma while axons are unresponsive¹¹. Systemic administration of kainic acid, a rigid analogue of glutamate with potent neuroexcitatory effects¹², causes degeneration of central nervous system (CNS) neurones in adult mice; but the attendant high incidence of seizures and death has limited its experimental use¹³. Intracerebral application of excitatory amino acids, however, induces localised neuronal degeneration with neuropathological characteristics similar to that resulting from peripheral administration^{14,15}.

As the sensitivity of neurones to the toxic effects of neuroexcitatory amino acids may be limited to soma and dendrites, it seemed that an intracerebral injection of kainic acid might cause degeneration of neurones with cell bodies near the injection site, but spare axons terminating in or passing through the area. We have tested the striatum because of its defined neuroanatomy and neurochemistry; it receives a dense innervation from dopaminergic neurones with cell bodies localised in the substantia nigra whereas the striatum itself is populated primarily by small neurones of which cholinergic and GABAergic (gamma aminobutyric acid) neurones are a major component¹⁶. Accordingly, we have examined the effects of a stereotaxic infusion of kainic acid into the striatum on the neurochemical markers for the intrinsic cholinergic and GABAergic neurones and the dopaminergic terminals in the region.

After anaesthesia with Equi-Thesin (0.6 ml; Jenson-Salisbury Labs), Sprague-Dawley rats (160 g) were positioned in a David Kopf small animal stereotaxic apparatus; and a 0.3-mm Hamilton cannula was inserted into the striatum through a burr hole in the calvarium (coordinates: 7.9 A; 2.6 L; 4.8 V). Drugs dissolved in 1 μ l of artificial cerebrospinal fluid (CSF) (titrated to pH 7.4) were infused over 1 min; the cannula was then removed and the scalp

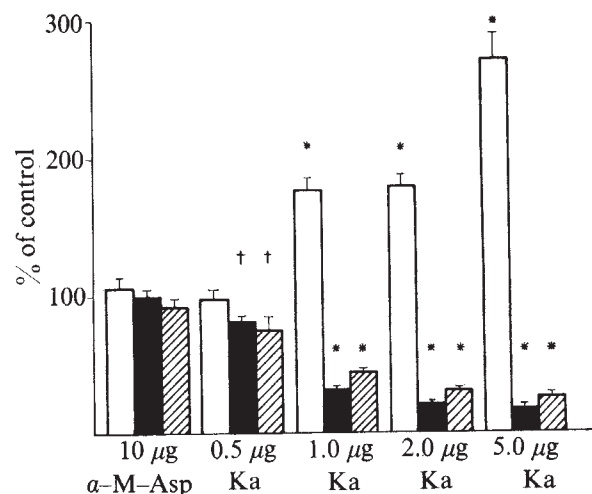


Fig. 1 The activities of tyrosine hydroxylase²³ (open bar), choline acetyltransferase²⁴ (solid bar) and glutamic acid decarboxylase²⁵ (striped bar) in the striatum were assayed 48 h after unilateral injection of various amounts of kainic acid (Ka) or α -methylaspartate (α -M-Asp). Results are presented in terms of percentage of the specific activity of the contralateral striatum with s.e.m. indicated. $N = 5$. * $P < 0.001$. † $P < 0.05$.

was apposed with sutures. At various times after treatment, the rats were killed; and the striata were dissected according to the method of Glowinski and Iversen¹⁷.

In initial experiments, the effects of infusion of various amounts of kainic acid (0.1–10 μ g) on the neurotransmitter-synthesising enzymes in the striatum were examined 48 h after injection (Fig. 1). With the injection of 0.5 μ g, there was a 20% reduction in the activities of glutamic acid decarboxylase (GAD) and choline acetyltransferase (CAT). With increasing doses, there was a progressive reduction in the activities of GAD and CAT whereas the activity of tyrosine hydroxylase (TH) exhibited a dose-dependent increase. Two days after injection of 2 μ g of kainic acid, the activities of GAD and CAT were reduced 80% whereas the activity of TH was increased 80%. Doses in excess of 5 μ g of kainic acid did not further alter enzyme activities in the striatum but were associated with a high rate of mortality. These changes in enzyme activity were not caused by the injection procedure, for the infusion of 10 μ g of α -methylaspartate, an analogue of glutamate without neuroexcitatory activity¹⁸, and did not alter significantly the activities of these enzymes. In contrast to the effects of kainic acid, the infusion of copper sulphate (25 μ g), a nonspecific toxic agent¹⁹, caused a similar 60–65% reduction in the activities of TH as well as GAD and CAT.

It is possible that the 80% decrease in the activities of CAT and GAD and 80% increase in the activity of TH after injection of 2 μ g of kainic acid reflected merely a transient neurochemical adaptation to excessive stimulation of glutamate receptors. To obtain further information, we examined several neurochemical markers for the cholinergic, GABAergic and dopaminergic neurones 10 d after infusion of 2.5 μ g of kainic acid (Table 1). The injection elicited a 60–70% decrement in the activity of GAD, the levels of endogenous GABA, the activity of the synaptosomal high affinity uptake process for GABA. Similarly, the activity of CAT, the levels of endogenous acetylcholine and the activity of the synaptosomal uptake process for choline were reduced 60–70%. In contrast, the activity of TH was 50% above control; the levels of endogenous dopamine and the activity of the synaptosomal uptake process for dopamine were not significantly affected by the injection. In addition, the wet weight of the striatum and protein content were not changed by the treatment.

Table 1 Effects of kainic acid on neurochemical parameters of the striatum 10 d after injection

		Injected	Control	Δ
GABAergic neurones				
Glutamic acid decarboxylase	nmol mg ⁻¹ h ⁻¹	4.13 ± 0.8	12.9 ± 0.9	-68*
GABA	nmol mg ⁻¹	0.72 ± 0.15	2.16 ± 0.17	-67*
GABA uptake	pmol per mg per 4 min	7.1 ± 1.2	16.5 ± 1.3	-57*
Cholinergic neurones				
Choline acetyltransferase	nmol mg ⁻¹ h ⁻¹	6.0 ± 1	20 ± 1	-70*
Acetylcholine	pmol mg ⁻¹	20 ± 4	68 ± 4	-71*
Choline uptake	pmol per mg per 4 min	0.05 ± 0.01	0.12 ± 0.01	-60*
Dopaminergic neurones				
Tyrosine hydroxylase	pmol mg ⁻¹ hr ⁻¹	340 ± 25	232 ± 21	+47*
Dopamine	pmol mg ⁻¹	52 ± 4	60 ± 4	-13
Dopamine uptake	pmol per mg per 4 min	3.1 ± 0.3	2.4 ± 0.3	+29
Wet weight	mg	48 ± 2	49 ± 2	-2
Protein	µg per mg tissue	165 ± 9	173 ± 7	-5

Kainic acid (2.5 µg) was injected into the striatum 10 d before killing the rats. The activities of tyrosine hydroxylase²³, choline acetyltransferase²⁴, and glutamic acid decarboxylase²⁵ and the levels of GABA²⁶, acetylcholine²⁷, dopamine²⁸ and protein²⁹ were assayed. The uptake of labelled GABA³⁰, choline³¹, and dopamine³² was measured in washed P₂ fractions prepared from striatum. The contralateral uninjected striatum served as control $N = 6-12$.

* $P < 0.001$.

Some of the GABAergic neurones with cell bodies in the striatum provide an inhibitory pathway innervating the dopaminergic cell bodies in the substantia nigra^{16,20}. In rats receiving a unilateral infusion of 2.5 µg of kainic acid in the striatum 1 week before death, the levels of endogenous GABA were reduced $67 \pm 3\%$ ($N=5$; $P<0.001$) in the ipsilateral substantia nigra. In contrast, the activity of tyrosine hydroxylase was not significantly altered in the substantia nigra on the injected side. Thus, the striatonigral GABAergic pathway seems to be affected selectively by the striatal injection of kainic acid.

Although these marked neurochemical alterations 10 d after injection of kainic acid suggest specific degeneration of cholinergic and GABAergic neurones intrinsic to the striatum, histological evidence is required. Therefore histological sections stained with cresyl violet were prepared from whole forebrain at the level of the striatum of animals

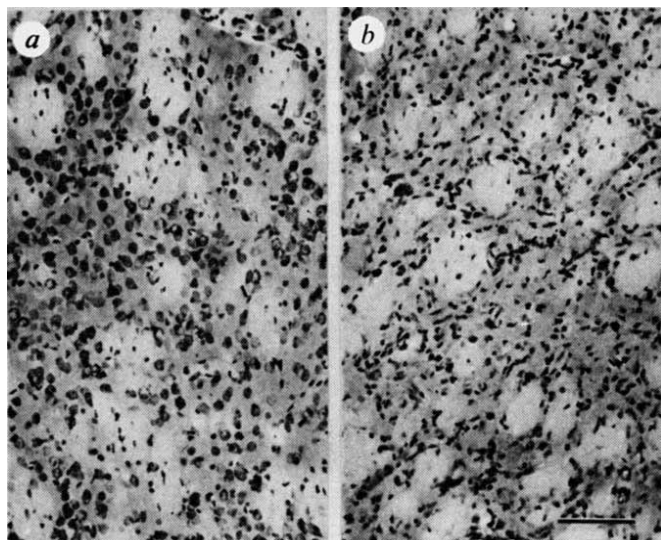
injected unilaterally with 2.5 µg of kainic acid 10 d before death (Fig. 2). Around the site of injection there was generalised loss of approximately 95% of neuronal perikarya in a radius of 1.5 mm, with a gradation in loss with increasing distance. Thus, the lesion involved nearly the entire head of the caudate putamen but did not affect the tail of the caudate or the globus pallidus (our work in preparation). Although the needle track was visible, there was no evidence of inflammatory changes or necrosis at the injection sites, but there was a generalised increase in the number and prominence of glia. The overall structure of the striatum containing the bundles of internal capsule fibres seemed to be unchanged except for the loss of neurones.

The striking behaviour of animals treated with a unilateral injection of kainic acid in the striatum demonstrates the physiological significance of the lesion. For up to 24 h after injection, the rats exhibited marked rotatory behaviour away from the side of the injection, which would be consistent with the neuroexcitatory effects of the drug in the striatum¹⁵. Two days after injection, however, they reversed the direction of rotation towards the side of the injection. The ipsilateral rotatory behaviour, which persisted up to 10 d after injection, is similar to that produced by lesions in the dopaminergic nigrostriatal pathway²¹. Thus, the lesion caused by kainic acid in striatal neurones, which are presumably postsynaptic to the dopaminergic terminals^{16,22}, results in a behaviour similar to that induced by ablation of the dopaminergic innervation to the striatum.

The striatal lesions produced by kainic acid have particular relevance to Huntington's chorea because the major site of neuropathological change in this disorder is the striatum, where a marked loss of intrinsic neurones and gliosis occurs¹. There are considerable decreases in the neurochemical markers for the cholinergic and GABAergic neurones in the striatum whereas the dopaminergic innervation to the region is relatively unaffected; in addition, there is a selective reduction in the activity of GAD and levels of GABA in the substantia nigra²⁻⁵. The neurochemical, histological and behavioural changes resulting from striatal infusion with kainic acid are remarkably similar to those observed in Huntington's chorea. Thus, lesions of the rat striatum produced with kainic acid may provide a useful animal model for Huntington's chorea, in which the biochemical, electrophysiological and pharmacological sequelae of the loss of striatal cholinergic and GABAergic neurones can be examined.

We thank Robert Zaczek for technical assistance and Dr Michael Kuhar and Naomi Taylor for doing the histological

Fig. 2 Micrographs of rat striatum showing the loss of neurones on the side injected with 2.5 µg of kainic acid (*b*) compared with the contralateral side (*a*) as control. Ten days after injection, the animals were killed by aortic perfusion with buffered formaldehyde. Sections (30 µm) were stained with cresyl violet. Neuronal perikarya are markedly reduced in number on the injected side which also shows marked gliosis. The lighter areas, which are bundles of internal capsule fibres, are unaffected by the treatment. The bar represents 100 µm.



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Evidence for a role of central serotonergic neurones in digitalis-induced cardiac arrhythmias

EVIDENCE indicates that digitalis drugs administered intravenously increase central sympathetic outflow and that this in turn results in cardiac arrhythmias^{1–6}. The central nervous system transmitter(s) that mediates this effect is not known. According to Saito *et al.*, noradrenaline, the primary transmitter studied so far, is not involved in the case of guinea pigs⁷. 5-Hydroxytryptamine (5-HT) however, may be an important chemical mediator regulating central sympathetic outflow^{8,9}, and it is involved in the respiratory arrest induced in rats by intravenous administration of digitoxigenin¹⁰. We now report evidence that suggests that brain 5-HT is involved in digitalis-induced cardiac arrhythmias.

Cats (1.6–3.6 kg) were anaesthetised with alpha chloralose (70–80 mg kg⁻¹ intravenously) and artificially ventilated with room air. The femoral artery and vein were catheterised for recording blood pressure and administering drugs, respectively. Rectal temperature was maintained between 36° and 38 °C by warming the cat with radiant heat. The electrocardiogram and the arterial blood pressure were recorded. The digitalis preparation, deslanoside, was administered by a continuous intravenous infusion of 2 µg kg⁻¹ min⁻¹. Doses necessary to produce the following endpoints were obtained: ventricular arrhythmia, ventricular tachycardia and ventricular fibrillation. The role of brain 5-HT in the arrhythmogenic effects of deslanoside was assessed by determining: (1) the effect of pretreatment with p-chlorophenylalanine (PCPA) on the doses of deslanoside

needed to produce ventricular arrhythmias (PCPA was administered as 300 mg kg⁻¹ intraperitoneally each day for 3 d before the animals were intoxicated with deslanoside); (2) the effect of 5,7-dihydroxytryptamine (5,7-DHT) on the doses of deslanoside needed to produce ventricular arrhythmias (5,7-DHT was administered as a single dose of either 100 µg kg⁻¹ to three cats, or 200 µg kg⁻¹ to two cats, into the anterior horn of the left lateral ventricle 8 d before intoxication with deslanoside), and (3) the effect of methysergide as both pretreatment (3 mg kg⁻¹ intravenously, 10 min before deslanoside infusion and repeated 45 and 85 min after deslanoside infusion started) and as a treatment (3 mg kg⁻¹ intravenously, 1–6 min after a ventricular arrhythmia had been evoked by deslanoside).

To assess the extent and selectivity of the depletion of brain 5-HT by PCPA and 5,7-DHT, the brains of cats intoxicated with deslanoside were removed immediately after the onset of ventricular fibrillation. The brains were placed on ice and the medulla-pons, hypothalamus and superior and inferior colliculi were dissected out, frozen on dry ice and stored at –20 °C until assayed (up to 3 weeks). Biogenic amines, 5-HT and noradrenaline were assayed by a modification of the procedures of Chang *et al.*¹¹ and Maickel *et al.*¹², as described in detail by Zenker *et al.*¹³. Results are expressed as the mean µg of amine per g of tissue ±s.e.m.

Fig. 1 Effect of methysergide on deslanoside-induced ventricular arrhythmia. *a*, Electrocardiogram (ECG) and arterial blood pressure (BP) tracings before intoxication with deslanoside. *b*, Effects of a cumulative dose of deslanoside (200 µg kg⁻¹) on ECG and BP just before the development of a ventricular arrhythmia. *c*, Deslanoside-induced ventricular arrhythmia. *d*, ECG and BP tracings 2.5 min after 3 methysergide (3 mg kg⁻¹) was administered.

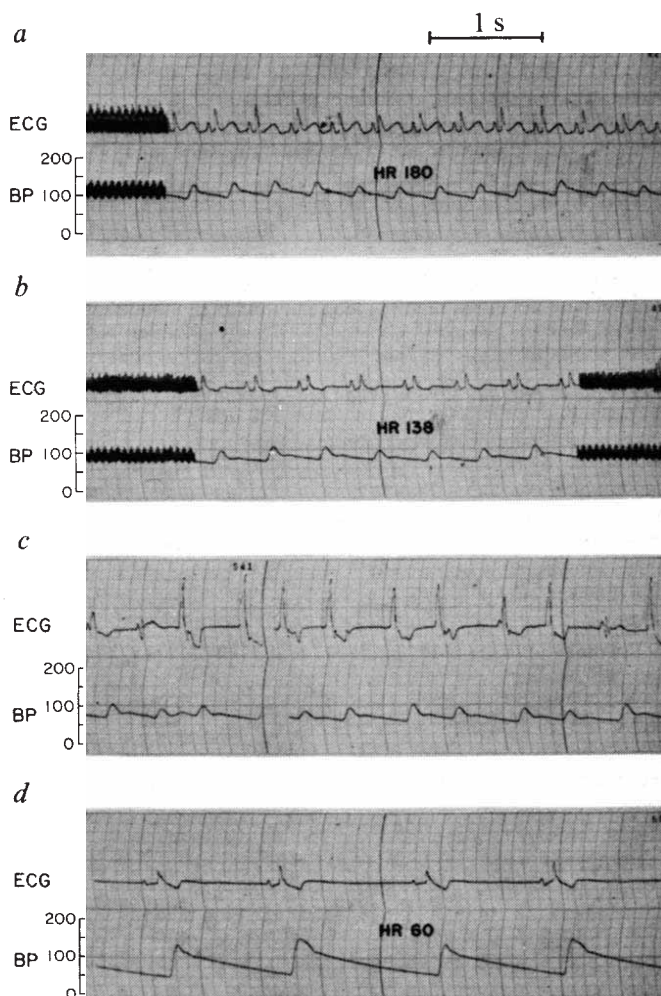


Table 1 Effect of *p*-chlorophenylalanine, 5,7-DHT and methysergide pretreatment on the capacity of deslanoside to produce ventricular arrhythmias

Group	No. in each group	Heart rate and blood pressure before deslanoside infusion		Doses of deslanoside to produce cardiac arrhythmias ($\mu\text{g kg}^{-1}$)		
		Heart rate (beats per min)	Mean blood pressure (mmHg)	Ventricular arrhythmia	Ventricular tachycardia	Ventricular fibrillation
Control	21	193 $\pm$ 5.8	136 $\pm$ 7.3	165 $\pm$ 8.5	178 $\pm$ 8.5	212 $\pm$ 11.3
<i>p</i> -Chlorophenylalanine pretreatment	7	186 $\pm$ 9.8	132 $\pm$ 7.8	238 $\pm$ 22.8*	246 $\pm$ 22.7*	277 $\pm$ 18.6*
5,7-Dihydroxytryptamine pretreatment	5	190 $\pm$ 6.4	123 $\pm$ 16.4	219 $\pm$ 10.6*	223 $\pm$ 10.4*	258 $\pm$ 15.1
Methysergide pretreatment	5	131 $\pm$ 12.3*	96 $\pm$ 7.7*	232 $\pm$ 29.0*	249 $\pm$ 27.5*	317 $\pm$ 35.4*

Controls were 12 animals given deslanoside only, three given saline intraperitoneally for 3 d and then intoxicated with deslanoside (to control for *p*-chlorophenylalanine injections), and six given saline into the anterior horn of the left lateral ventricle and 8 d later intoxicated with deslanoside (to control for the 5,7-DHT injection). Values between the three control groups were not significantly different so the data were pooled.

* $P < 0.05$ when data are compared with corresponding control data.

of triplicate determinations. Using a Farrand Fluorescent MK-1 spectrophotofluorometer, the fluorescent intensity of the biogenic amines was determined in 1-cm quartz cells.

The pretreatment studies (Table 1) showed that animals given any one of the three agents that interfere with brain 5-HT function required a significantly larger dose of deslanoside to produce ventricular arrhythmias. PCPA and 5,7-DHT inhibit tryptophan hydroxylase and destroy central serotonergic nerves, respectively; animals pretreated with these drugs exhibited a significant decrease in the 5-HT content in all brain regions analysed (Table 2). Depletion

Taken together, these results suggest that central serotonergic neural systems are involved in the central arrhythmogenic effect of digitalis. Central serotonergic neural systems have been demonstrated by some investigators to have an important influence on central sympathetic outflow^{8,9,19}. For example, Wing and Chalmers⁹ reported that intracerebral administration of 5,6-DHT prevents the increases in arterial blood pressure and heart rate produced by sinoaortic denervation in unanaesthetised rabbits. This suggests that activity in central serotonergic nerves increases central sympathetic outflow. Wing and Chalmers did not

Table 2 Effect of *p*-chlorophenylalanine and 5,7-DHT on 5-HT and noradrenaline content of selected brain regions

Group	No. in each group	Medulla-pons		Brain region Hypothalamus		Colliculi	
		5-HT ($\mu\text{g g}^{-1}$)	Noradrenaline ($\mu\text{g g}^{-1}$)	5-HT ($\mu\text{g g}^{-1}$)	Noradrenaline ($\mu\text{g g}^{-1}$)	5-HT ($\mu\text{g g}^{-1}$)	Noradrenaline ($\mu\text{g g}^{-1}$)
Controls (no drug)	6	0.73 $\pm$ 0.03	0.91 $\pm$ 0.05	1.53 $\pm$ 0.04	1.30 $\pm$ 0.04	0.87 $\pm$ 0.03	0.28 $\pm$ 0.02
Animals intoxicated with deslanoside	6	0.75 $\pm$ 0.03	0.85 $\pm$ 0.04	1.53 $\pm$ 0.04	1.20 $\pm$ 0.04	0.86 $\pm$ 0.03	0.31 $\pm$ 0.03
Animals pretreated with <i>p</i> -chlorophenylalanine and intoxicated with deslanoside	3	0.28 $\pm$ 0.04*	0.87 $\pm$ 0.03	0.22 $\pm$ 0.02*	1.32 $\pm$ 0.04	0.06 $\pm$ 0.02*	0.29 $\pm$ 0.03
Animals pretreated with 5,7-DHT and intoxicated with deslanoside	5	0.51 $\pm$ 0.05*	0.86 $\pm$ 0.05	0.83 $\pm$ 0.16*	1.39 $\pm$ 0.05	0.43 $\pm$ 0.11*	0.29 $\pm$ 0.03

* $P < 0.05$ when data are compared with either the control group or the animals intoxicated with deslanoside.

of 5-HT content with these drugs seemed to be selective, as no significant changes in noradrenaline were observed.

Deslanoside had no significant effect on brain 5-HT levels (Table 2). This was not unexpected, for treatments that enhance central serotonergic neural activity do not alter 5-HT levels although they increase turnover¹⁴.

We tested methysergide for antiarrhythmic effects because this drug rapidly antagonises 5-HT receptors after intravenous administration¹⁵. It was administered intravenously (as a bolus of 3 mg kg⁻¹) to three control cats with a sustained ventricular tachycardia induced by deslanoside. In each cat, methysergide converted the existing ventricular arrhythmias to sinus rhythm (Fig. 1).

Pretreatment with PCPA and 5,7-DHT had no significant effect on baseline heart rate and arterial pressure. Pretreatment with methysergide resulted in a significant reduction in both heart rate and arterial pressure (Table 1).

We have also found¹⁶ that doses of deslanoside that produce lethal ventricular arrhythmias significantly increase 5-hydroxyindoleacetic acid tissue concentration and tryptophan hydroxylase activity in the hypothalamus, amygdala and colliculi. This indicates that deslanoside produces biochemical changes in the central serotonergic system that are consistent with the drug activating serotonergic neurones¹⁷. We have also found¹⁸ that electrical stimulation of midbrain raphe areas causes similar kinds of arrhythmias and biochemical changes (changes in 5-hydroxyindoleacetic acid and tryptophan hydroxylase activity) as does deslanoside.

observe drastic changes in baseline heart rate or arterial pressure with 5,6-DHT, indicating that normal sympathetic outflow may not be greatly affected by depleting brain 5-HT. In the study reported here, no significant changes in these baseline cardiovascular parameters were observed with either 5,7-DHT or PCPA. Thus interference with central 5-HT synthesis or selective degeneration of central 5-HT nerve endings may prevent noxious influences (digitalis overdose or sinoaortic denervation) from deranging central sympathetic outflow without disrupting normal tonic central sympathetic activity.

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Absolute sensitivity of rod bipolar cells in a dark-adapted retina

THE visual system of vertebrates mediated by rods is capable of detecting a few quanta of light^{1,2}. The work reported here is concerned with the voltage response to flashes of light in individual bipolar cells, to which the rods send their signals and which, in turn, signal to amacrine and ganglion cells. We measured the absolute sensitivity of rod bipolar cells in the dogfish retina. The photoisomerisation of a single rhodopsin chromophore in only one rod out of 100 leads to a response of 1 mV in the bipolar cell.

Intracellular recordings were made in the eyecup preparation of the dogfish, *Scyliorhinus canicula*, dissected in dim red light from animals dark-adapted for at least 3 h. This species has visual receptors which are almost exclusively rods, containing a photopigment³ with maximum absorbance at 500 nm. The light source was a tungsten-iodine lamp operated from a stabilised d.c. supply and filtered by a bandpass interference filter with peak transmission at 495 nm. The irradiance of the source through the bandpass and heat-absorbing filters was calibrated with a radiometer (Tektronix J16/J6502 radiance probe), and checked by a photomultiplier in single-photon counting mode. Dark adaptation was maintained by using dim test flashes to locate bipolar cells. Cells were penetrated in the region of retina backed by the tapetum lucidum. The responses reported here were obtained with full-field illumination.

The identification of the cells as bipolar cells is based on criteria of depth, waveform and receptive field. The cells were

Fig. 1 Responses of a bipolar cell in the dark-adapted retina of the dogfish *Scyliorhinus canicula* to 18-ms flashes of light of varying intensity. An increase of internal potential (depolarisation) is indicated by an upward deflection. The timing of the flash is shown beneath the superimposed traces. The numbers near each trace represent the number of photons μm^{-2} at 495 nm delivered by the flash. The internal potential of the cell in the dark was -40 mV.

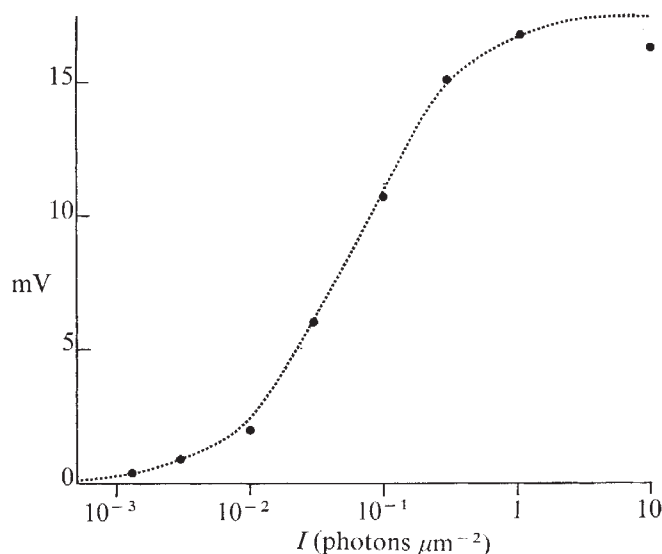
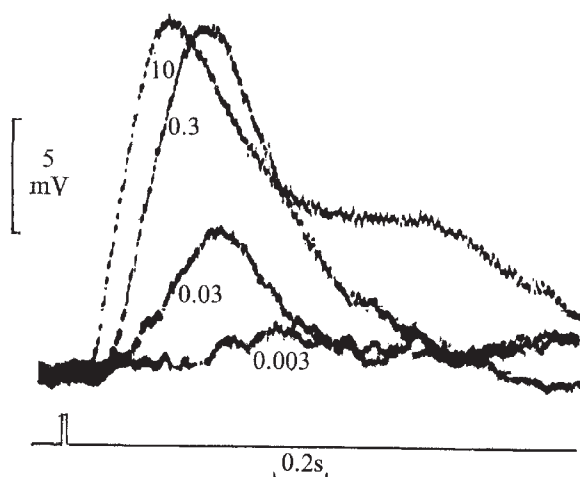


Fig. 2 The relationship between light intensity and peak of the response for the bipolar cell shown in Fig. 1. Note that the scale for light intensity is logarithmic. The three points shown at the lowest light levels were obtained by signal averaging, each point being the average of eight responses.

impaled at depths of 70–100 μm from the vitreal surface of the retina, often in close proximity to a horizontal cell. The response to light flashes (Fig. 1) consists of a depolarisation graded with the light intensity, with a maximum response of up to 30 mV. The response characterised by a phasic peak, which declines to a plateau, is evoked at higher light intensities. This is the response described by Kaneko for bipolar cells, identified by staining with Procion dyes in the retina of the related species, *Mustelis canis*⁴. As in bipolar cells in other retinæ, there is an 'off' response which follows the cessation of a long, near-saturating pulse of light⁵. The receptive field for the centre response of these cells is less than 500 μm and in some cells less than 200 μm . An antagonistic effect produced by illumination of the surround is often used to identify bipolar cells; the cells reported here, however, have only a weak centre-surround organisation. There are reasons to suppose that dark adaptation diminishes the effect of the surround⁶.

To analyse the sensitivity of the cells, the responses to brief flashes of light were recorded at low light levels in the region over which the response was proportional to intensity, as well as at higher light levels. The small signals evoked by dim lights were averaged by means of a computer. Figure 2 shows the intensity-response curve for the cell whose responses were illustrated in Fig. 1. The line of Fig. 2 fitted to the experimental points is of the form

$$V = V_{\max} \frac{I}{I + I_{1/2}} \quad (1)$$

a hyperbolic relationship between the response, V , and the light intensity, I . For this cell $V_{\max} = 17.5$ mV and the half-saturating intensity, $I_{1/2} = 0.06$ photons μm^{-2} per flash. The absolute sensitivity of the cell⁷ was determined as the ratio of response to intensity of light, within the range over which the response was proportional to light intensity. The flash sensitivity for this cell was 310 mV photon⁻¹ μm^2 . The results for other bipolar cells are given in Table 1. There was considerable variation in sensitivity and $I_{1/2}$ values among cells, but the flash sensitivity consistently exceeded 100 mV photon⁻¹ μm^2 , provided that $V_{\max}$ was greater than 8 mV.

To convert these figures to the voltage response that would be produced by the photoisomerisation of a single rhodopsin

Table 1 Flash sensitivity of rod bipolar cells and the semi-saturation value of light intensity, $I_{1/2}$

$V_{\max}$ (mV)	Flash sensitivity (mV photon ⁻¹ μm^2)	Flash sensitivity (mV rod per isomerisation)	$I_{1/2}$ (isomerisation per rod)
17.5	310	103	0.18
23.8	330	110	0.42
21.8	200	67	0.33
14.1	373	124	0.55
13.8	137	46	1.20
25.5	170	57	0.63
8.1	476	159	0.18
9.3	228	76	0.55
18.5	323	108	0.19
Mean	283	95	0.47

For some cells, the slope of the intensity-response curve near $I_{1/2}$ was significantly less steep than would be indicated by equation (1). The extent of the deviation may be gauged by comparing the flash sensitivity derived from the response to dim flashes with the value $V_{\max}/I_{1/2}$. The internal potential of the cells in the dark ranged between -40 and -62 mV.

chromophore in each rod, an estimate of the photoisomerisation cross section (also called the effective collecting area⁷) is required. The geometric cross section of *Scyliorhinus* rod outer segments suspended in vitreal fluid is $7.1 \mu\text{m}^2$ and their length is $28 \mu\text{m}$. On the assumption of a specific axial density⁸ for rhodopsin of $0.014 \mu\text{m}^{-1}$, a quantum efficiency for photoisomerisation of 0.67 (ref. 9), a reflectivity of the tapetum of 90% (ref. 3) and the further assumption that all the incident light passes into the outer segments, the photoisomerisation cross section would be $3.7 \mu\text{m}^2$. This figure takes no account of the fact that some of the light will pass between photoreceptors as well as being scattered by other retinal layers. We therefore tentatively assign a value of $3 \mu\text{m}^2$ for the photoisomerisation cross section. This value has been used to derive the flash sensitivities of the rod bipolar cells (given as the response in mV per photoisomerisation in each rod) and to estimate the number of photoisomerisations per rod necessary for half saturation of the response of a bipolar cell. On average, the response is half-saturated when one rhodopsin chromophore is isomerised in just one out of two rods and there is a response of ~ 1 mV in the bipolar cell when there has been one photoisomerisation in one out of 100 rods (Table 1).

Because of their small size, it has not been possible to determine the flash sensitivity of dogfish rods. The highest values reported for flash sensitivities of uniformly illuminated rods were obtained by Fain¹⁰ in *Bufo marinus* (0.68 mV per photoisomerisation) and by Copenhagen and Owen¹¹ in the snapping turtle (0.72 mV per photoisomerisation). There is no reason to suppose that the rods of dogfish are unusually sensitive. Measurement of the externally recorded receptor potential of the electroretinogram yields a value for the number of photoisomerisations for half saturation similar to that reported for other vertebrate rods¹⁰⁻¹⁴. It is possible that the isomerisation of a chromophore in a dogfish rod may lead to a somewhat larger voltage change than has been observed in rods of larger diameter. If photoisomerisation produces the same underlying membrane conductance change in all rods, the voltage change produced would then depend on the dimensions of the cell, varying approximately as $1/\text{diameter}$ if the rod were regarded as a short cable. On this basis, the flash sensitivity for dogfish rods might be about 2 mV per photoisomerisation. If rods are electrically coupled^{10,11,15}, then at low light levels when only a few rods absorb a photon, the voltage response in any one rod will be only a small fraction of what would be obtained in an electrically independent rod.

These measurements allow some deductions about signal transmission between rods and bipolar cells to be made. The dynamic voltage gain in signal transmission is the ratio of the flash sensitivity of the bipolar cell to that of the rods; in this case, yielding a gain of about 50. Schwartz¹⁶ also concluded that there was a large increase in signal amplitude in transmission from cones to bipolar cells in the turtle retina. A dynamic voltage gain of 50 can be compared with a value of

about 3, applicable to a number of conventional chemical synapses¹⁷. In both cases, it must be assumed that the postsynaptic cell linearly sums the transmitter-modulated conductance increments contributed by all the presynaptic cells which converge on it. The maximum dynamic gain can be specified in terms of the fractional contribution of transmitter-modulated conductance to the total conductance of the postsynaptic cell (and, therefore, does not depend explicitly on convergence). The higher dynamic gain at the rod-bipolar cell synapse remains to be explained.

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Identification of actin-binding protein in membrane of polymorphonuclear leukocytes

A NEW protein with a high molecular weight and found in rabbit alveolar macrophages¹, causes actin filaments in sucrose solutions to form a solid gel when warmed to room temperature. With transmission electron microscopy, the actin filaments were observed to be bound into branching interconnecting bundles². The new protein was called actin-binding protein (ABP). Similar consistency changes, which are due to gelation of actin associated with other proteins, have been observed in sucrose extracts of *Acanthamoeba* and glycerol extracts of sea urchin eggs^{3,4}. ABP was subsequently isolated from extracts of chronic myelogenous leukaemia (CML) leukocytes⁵. The CML leukocyte ABP had identical properties to the analogous protein isolated from the rabbit alveolar macrophage. The CML leukocyte ABP in the presence of actin produced a gel that structurally resembled the hyaline ectoplasm of a pseudopod. Since particle contact with the phagocytic membrane leads

to generation of a pseudopod, it seemed reasonable that ABP might be associated with the membrane. Using an antibody directed against CML leukocyte ABP coupled with indirect immunological stains, we were able to localise ABP to the cytoplasmic surface of the membrane of normal polymorphonuclear leukocytes.

We prepared⁶ rabbit IgG against purified CML leukocyte ABP (Fig. 1). The antibody activity was eliminated by absorption with either purified ABP or intact normal polymorphonuclear (PMN) leukocytes. Furthermore, no cross reactivity was demonstrated in an Ochterlony plate with two different preparations of myosin antiserum. We had noted previously that ABP antibody inhibited the gelation of CML leukocyte extracts as well as of purified leukocyte actin filaments in the presence of ABP⁵.

In rabbit alveolar macrophages, contact of an ingestible particle with the cell surface generates more extractable ABP². These findings suggest that ABP might be associated with the plasma membrane of phagocytes. To verify whether human leukocyte ABP is found in PMN leukocyte membranes, we prepared phagolysosomes after allowing the cells to phagocytose opsonised lipopolysaccharide-coated paraffin oil droplets⁷. The phagolysosome forms by fusion of lysosomal granule membranes with inverted sections of the plasma membrane. These 'inside-out' membrane preparations were exposed to purified anti-ABP IgG or normal rabbit IgG and then treated with goat anti-rabbit IgG (γ chain) conjugated with fluorescein isothiocyanate (FITC; Hyland Laboratory, Costa Mesa, California). Diffuse membrane fluorescence was observed with the anti-ABP IgG (Fig. 2). Since the plasma membrane Fc receptors were hidden inside these phagolysosomes, there was no observable fluorescence with normal rabbit IgG. Staining was also eliminated from ABP antibodies by absorbing the antibody with purified ABP or intact PMN leukocytes.

The nonspecific fluorescence of intact cells prevented us from using the same technique to identify ABP on their plasma membranes. For this study, we used an immunoperoxidase stain⁸ which eliminated the problem. Peripheral

Fig. 1 Immunodiffusion of human leukocyte ABP (centre well) against anti-ABP IgG (1) and against 1:2 dilution of the anti-ABP IgG (4); two different anti-leukocyte myosin antisera (5 and 6); anti-ABP antisera absorbed with intact PMN leukocytes (2); and anti-ABP antisera absorbed with purified ABP antigen (3).

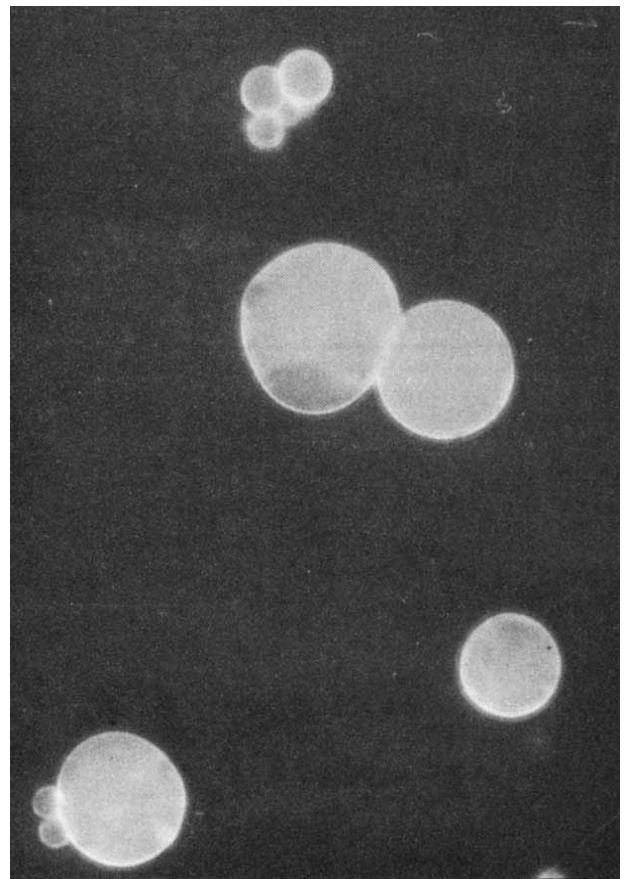
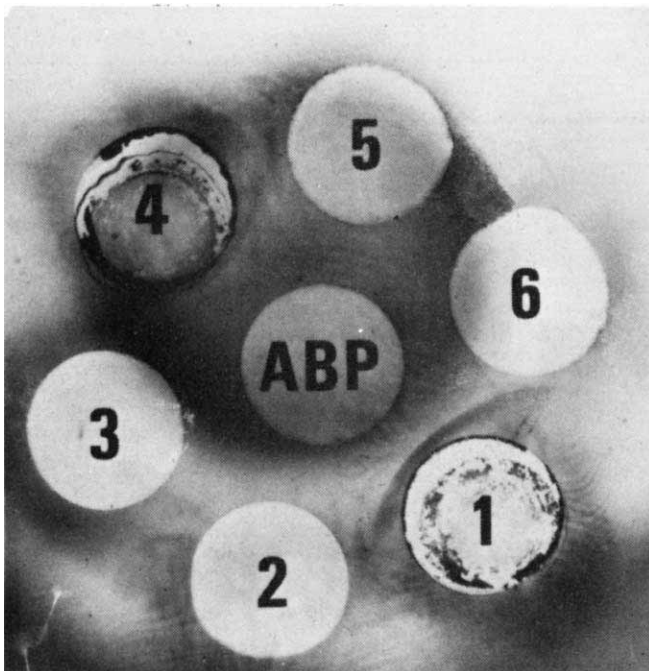
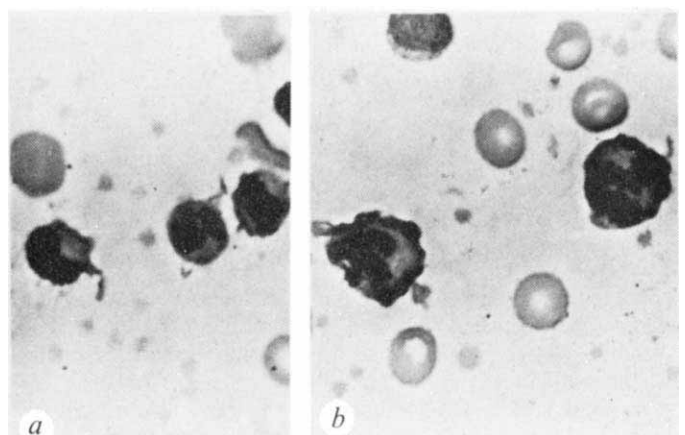


Fig. 2 Phagocytic vesicles were incubated with ABP IgG for 20 min at 25 °C. The vesicles were washed three times in Krebs-Ringer phosphate, pH 7.4, and suspended in goat anti-rabbit IgG (γ chain) conjugated with fluorescein for 20 min; then they were washed again. The resulting phagocytic vesicle was observed after settling on to a glass coverslip using a Zeiss epiilluminator with a FITC interference filter, 450 dichroic mirror, 53 barrier filter, and a $\times 100$ objective.

blood leukocytes were treated with peroxide and methanol to block endogenous peroxidase. The cells were incubated with anti-ABP IgG, washed, and incubated with goat anti-rabbit IgG conjugated with horseradish peroxidase and washed again. The fixed dried smears were then stained for peroxidase. Those cells incubated with anti-ABP IgG had maximal reactivity at the cell margins (Fig. 3). No

Fig. 3 Peripheral blood cells stained with normal rabbit IgG (a) and with anti-ABP IgG (b). Note the positive reaction at the periphery of the cells.



staining of PMN leukocytes occurred with a normal IgG nor of lymphocytes or monocytes with the specific antibody.

Actin, myosin and ABP have been identified by polyacrylamide gel electrophoresis in enriched rabbit alveolar membrane fractions⁹. Whether ABP is an integral protein or largely associated with surfaces of the leukocyte membrane is not ascertainable from our studies. It is, however, tempting to believe that ABP by its membrane location could transduce surface recognition of particles into mechanical events, leading to actin polymerisation and the generation of pseudopodia.

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Transient kinetics of $(\text{Na}^+ + \text{K}^+)$ -ATPase studied with a fluorescent substrate

We describe experiments, with a purified $(\text{Na}^+ + \text{K}^+)$ -ATPase, designed to investigate details of the enzymatic process that couples ATP hydrolysis to the active transport of Na^+ and K^+ ions. We have found that formycin triphosphate^{1–3} (FTP), an analogue of ATP previously used in studies of myosin ATPase⁴, is a substrate for the $(\text{Na}^+ + \text{K}^+)$ -ATPase, and that the binding of FTP or of formycin diphosphate (FDP) to the enzyme is accompanied by a two- to threefold enhancement of nucleotide fluorescence. The change in strength of the fluorescent signal has allowed us to measure equilibrium binding of the nucleotides to the enzyme, and using stopped-flow fluorimetry we have been able to measure the rates of binding and release. FTP and FDP bind with a high affinity and may be displaced by excess ATP or by K^+ ions. During turnover, at least with FTP concentrations up to $24 \mu\text{M}$, the FDP is released (or its fluorescence is quenched) before the rate-limiting step of the overall reaction, both in the presence and absence of K^+ ions. When turnover is prevented by the absence of magnesium, the enzyme is still able to change its conformation according to whether Na^+ or K^+ is the predominant alkali-metal ion⁵. Because the Na and K conformations have different nucleotide affinities, we have been able to measure the rates of the conformational changes by monitoring the decrease or increase in fluorescence associated with the net release or binding of nucleotide. Finally, the effects of alterations in FTP concentration on the time course of the fluorescence changes have provided a clue to the non-phosphorylating role of ATP.

All experiments were done at about 21°C with a $(\text{Na}^+ + \text{K}^+)$ -ATPase from pig kidney, purified by the simpler method of Jørgensen⁶. The enzyme had a specific activity of about $17 \mu\text{mol}$

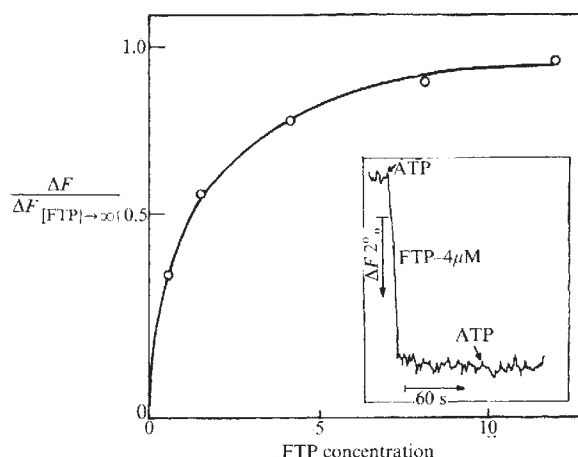


Fig. 1 Binding of FTP to $(\text{Na}^+ + \text{K}^+)$ -ATPase as a function of FTP concentration. $(\text{Na}^+ + \text{K}^+)$ -ATPase prepared from pig kidney by the simpler procedure of Jørgensen⁶ was washed to remove traces of ATP and suspended at $60 \mu\text{g ml}^{-1}$ in 2.5 ml of a solution containing NaCl 50 mM, EDTA (Tris salt, pH 7.7) 5 mM, and FTP (triethylamine salt) as indicated. ATP was added in $2 \mu\text{l}$ steps to give a final concentration of $25 \mu\text{M}$. Fluorescence was measured at room temperature in a Farrand fluorimeter modified to allow additions to be made during continuous stirring and recording. Fluorescence was excited at 310 nm, and the light emitted at 380 nm was measured using 10-nm slits. A Wratten 18A stray-light filter was placed after the excitation monochromator, and a Schott W.G.365 stray-light filter before the emission monochromator. With $4 \mu\text{M}$ FTP, about half of the emitted light came from intrinsic protein fluorescence and scattering. Inset: reduction in fluorescence (ΔF) produced by the addition of ATP. Note that a second addition caused no further change, and that with $4 \mu\text{M}$ FTP, ΔF was only about 4% of total 380-nm emission, because the FTP initially bound to the enzyme was only a small fraction (2–3%) of the total FTP in the cuvette.

ATP hydrolysed per mg protein min^{-1} at 37°C , and well over 99% of the activity was sensitive to ouabain. FTP binding to the enzyme was measured by mixing FTP and enzyme in the fluorimeter cell, in the presence of EDTA, and then measuring the reduction in fluorescence when the bound nucleotide was displaced by a large excess of ATP. Measurements of binding at several FTP concentrations gave the saturation curve shown in Fig. 1 and a calculated dissociation constant of $1.1 \mu\text{M}$. A similar experiment with FDP gave a dissociation constant of $4.8 \mu\text{M}$. ATP competed effectively with both FTP and FDP, and the calculated inhibition constant was $0.15 \mu\text{M}$, which is similar to published values of $0.22 \mu\text{M}$ (ref. 7) and $0.12 \mu\text{M}$ (ref. 8) for the ATP dissociation constant.

Fluorescence enhancement could also be reversed by the addition of K^+ , Ti^+ , Rb^+ , NH_4^+ and Cs^+ , almost certainly because these ions cause dissociation of bound nucleotide⁷. Figure 2 shows the results of an experiment in which FDP was displaced from the enzyme by titration with KCl; virtually identical results were obtained with FTP. In the presence of $133 \mu\text{M}$ Na^+ , 50% dissociation of nucleotide was obtained with a K^+ concentration of $140 \mu\text{M}$.

The kinetics of FTP binding and FDP release during enzyme turnover were studied with the stopped-flow fluorimeter. Enzyme from one syringe was mixed with Mg^{2+} and FTP from another, in the presence of Na^+ and Tris buffer. Figure 3 shows the results of two experiments with different time scales. Mixing was followed by (i) a very rapid rise in fluorescence, (ii) a rapid fall in fluorescence, with a rate constant of 14 s^{-1} in Fig. 3a, (iii) a period of low fluorescence, and (iv) a final rise in fluorescence. The initial very rapid rise in fluorescence cannot be seen in Fig. 3 because of the time constant chosen. It almost certainly represents FTP binding, and it gives a calculated second-order rate constant of $2\text{--}8 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$; the rate was too fast to allow more precise determination. The rapid drop in fluorescence is most simply attributed to FDP release from

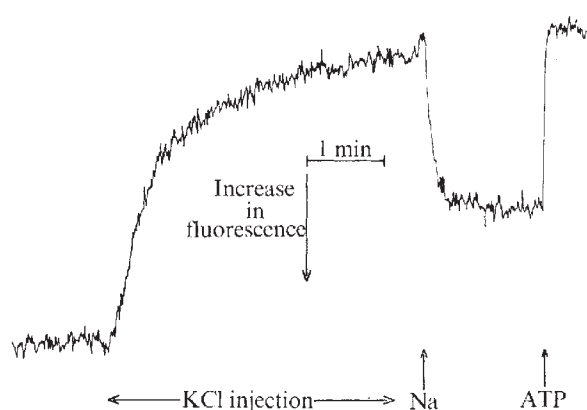
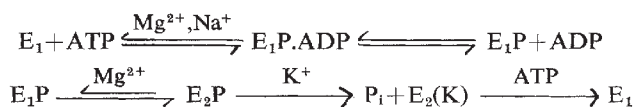


Fig. 2 Dissociation of FDP from $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$ by the addition of K^+ ions. The fluorimeter cell contained enzyme suspended at a concentration of $153 \text{ mg protein ml}^{-1}$ in a solution containing FDP (triethylamine salt) $4 \mu\text{M}$; Tris/Tris Cl (pH 7.7) 80 mM ; EDTA (Tris salt) 1 mM ; NaCl $133 \mu\text{M}$. KCl was added from a motor-driven Agla syringe which delivered $5 \mu\text{l}$ of 150 mM KCl per minute. The syringe was stopped after 4 min, by which time the K^+ concentration was 1 mM . NaCl, to a final concentration of 17 mM , and ATP, to a final concentration of $27 \mu\text{M}$, were added by hand. FDP fluorescence was excited at 310 nm and viewed at 380 nm as described in the legend to Fig. 1. An upward displacement of the trace represents a decrease in fluorescence and, because the rate of addition of KCl was very slow, the trace shows a succession of equilibrium states and gives no information about the rate of change of the enzyme (compare Fig. 4b).

the enzyme, though it could conceivably have been caused by a reduction in the fluorescence of bound FDP. The period of low fluorescence continued during hydrolysis of the FTP in the medium, and probably reflects the existence of a steady-state complex with a low affinity for nucleotide. The final rise in fluorescence may be attributed to binding of FDP from the medium as the concentration of FTP fell below its dissociation constant and the steady-state complex disappeared.

The presence of 1 mM K^+ , added with the FTP, did not significantly alter the rate of fall of fluorescence (reflecting FDP release), though it did prolong the period of low fluorescence. The lack of effect of K^+ ions on the rate of fall of fluorescence is consistent with the Albers-Post sequence (see ref. 9), namely



where E_1 is a form of the enzyme, with a high affinity for ATP, which exists in predominantly Na media, E_1P is a phosphorylated form that can transfer its phosphoryl group to ADP, E_2P is a phosphorylated form that is hydrolysed slowly spontaneously, but rapidly in the presence of K^+ ions, and $\text{E}_2(\text{K})$ is a form of the enzyme (containing occluded K^+) that is produced by K^+ -catalysed hydrolysis of E_2P and is converted to E_1 by a reaction which is accelerated by the binding of ATP at a low-affinity site. The low level of fluorescence during steady-state hydrolysis, both in the presence and absence of K^+ ions, also supports this scheme, by providing evidence that the release of ADP precedes the step that is rate limiting—presumably E_2P hydrolysis if K^+ ions are absent and $\text{E}_2(\text{K})$ breakdown if they are present. The prolongation of the period of low fluorescence in the presence of K^+ is simply the result of the reduction in the overall hydrolysis rate, which occurs because the breakdown of $\text{E}_2(\text{K})$ at these very low nucleotide concentrations is slower than the spontaneous hydrolysis of E_2P .

A series of experiments like that of Fig. 3b, but with different FTP concentrations, showed that the fall in fluorescence was progressively accelerated as the FTP concentration was increased

in the range $2\text{--}20 \mu\text{M}$, beyond which measurements were impracticable.

We have also used changes in the affinity for FTP and FDP to investigate the rates of interconversion of the dephosphoenzyme between a form (E_0) with a low affinity for nucleotide, which exists when K^+ is the predominant cation, and a form (E_1) with a high affinity for nucleotide, which exists when Na^+ is the predominant cation^{5,10-12}. Figure 4a shows the slow increase in fluorescence that occurred when enzyme preincubated with 0.8 mM K^+ in the absence of Na^+ was mixed with an equal volume of buffer containing $4 \mu\text{M}$ FTP and 100 mM Na^+ . No magnesium was present and the buffer concentrations were adjusted to minimise changes in ionic strength. Since the binding of FTP to enzyme preincubated with Na^+ is very rapid ($\sim 200 \text{ s}^{-1}$ at $4 \mu\text{M}$ FTP), the rate-limiting step in the experiment of Fig. 4a must have been the conversion of the K form to the Na form, or some process associated with that conversion.

In a series of similar experiments, increasing the FTP concentration in the range 4 to $24 \mu\text{M}$ increased the rate constant for the increase in fluorescence from ~ 0.25 to $\sim 0.85 \text{ s}^{-1}$. Again, this points to the existence of a low affinity FTP binding site, and it suggests that binding of FTP at the low affinity site accelerates the change in conformation.

Change in conformation in the reverse direction (Na form to K form) was monitored in experiments like that of Fig. 4b, in which enzyme that had been loaded with nucleotide in the presence of $300 \mu\text{M}$ Na^+ was suddenly exposed to 1 mM K^+ .

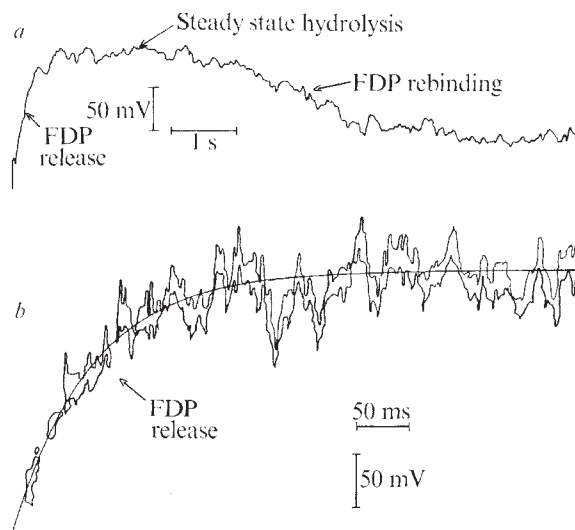


Fig. 3 Stopped-flow records of fluorescence changes accompanying FTP hydrolysis by $(\text{Na}^+ + \text{K}^+)\text{-ATPase}$. Experiments were done at $20\text{--}21^\circ\text{C}$ using a stopped-flow fluorimeter based on that described in ref. 14 but modified for split-beam operation. Fluorescence was excited at 310 nm , and the emitted light was detected after passage through Wratten 18A and Schott K.V.370 filters, which excluded most of the light below 360 nm . Note that an upward displacement of the trace represents a decrease in fluorescence. Syringe I contained: enzyme $300 \mu\text{g protein ml}^{-1}$; NaCl 76 mM ; Tris/Tris Cl (pH 7.7) 19 mM ; histidine 1.1 mM ; EDTA $40 \mu\text{M}$. Syringe II contained: FTP (triethylamine salt) $8 \mu\text{M}$ in Fig. 3a and $14 \mu\text{M}$ in Fig. 3b; MgCl_2 2.4 mM ; NaCl 76 mM ; Tris/Tris Cl (pH 7.7) 19 mM . The two syringes delivered equal volumes to the mixing chamber. a, Changes in fluorescence for a period of 10 s after mixing, recorded with a Datalab transient recorder. The time constant of the apparatus was set at 100 ms . b, Outline of an oscilloscope trace from a separate experiment showing changes in fluorescence for the first 0.5 s after mixing. The time constant was set at 5 ms . The continuous line represents the equation

$$y/y_\infty = 1 - \exp(-13.6t)$$

and is intended merely to show that, discounting noise, the fall in fluorescence can be described by a single exponential.

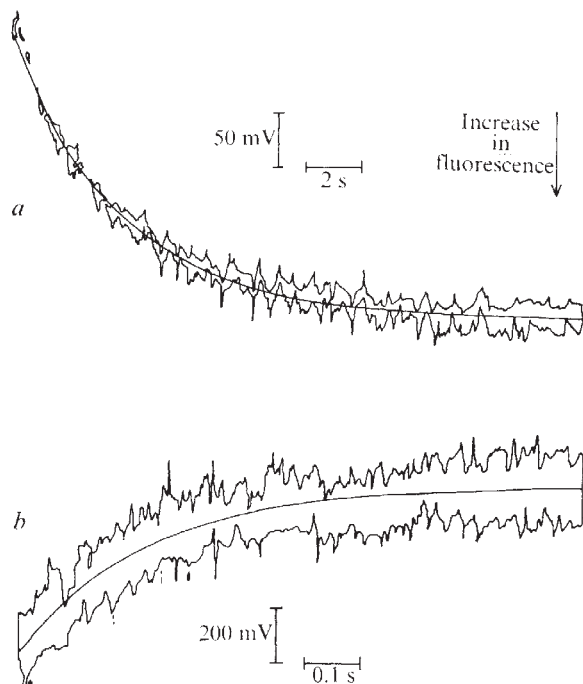


Fig. 4 Use of formycin nucleotides and the stopped-flow fluorimeter to follow slow conformational changes of the dephosphoenzyme induced by altering the concentrations of K^+ and Na^+ ions. *a*, Syringe I contained: enzyme 540 μ g protein ml^{-1} ; KCl 0.84 mM; Tris/Tris Cl (pH 7.7) 87 mM; histidine 4 mM; EDTA (Tris salt) 1 mM. Syringe II contained: FTP (triethylamine salt) 8 μ M; NaCl 100 mM; Tris/Tris Cl (pH 7.7) 4 mM. The addition of Na^+ and FTP to the K form of the enzyme produced a slow increase in fluorescence, presumably caused by binding of FTP to the Na form of the enzyme as soon as it appeared. The time constant of the amplifier was 50 ms. The figure reproduces the outline of the oscilloscope trace, and the smooth curve represents the equation

$$y = y_0 \exp(-0.26t)$$

b, Syringe I contained: enzyme 380 μ g protein ml^{-1} ; FDP (triethylamine salt) 4 μ M; NaCl 330 μ M; Tris/Tris Cl (pH 7.7) 100 mM; EDTA (Tris salt) 1 mM; histidine 4 mM. Syringe II contained: FDP (triethylamine salt) 4 μ M; KCl 2 mM; Tris/Tris Cl (pH 7.7) 100 mM; EDTA (Tris salt) 1 mM. The addition of K^+ to enzyme-FDP caused a slow fall in fluorescence, presumably the result of dissociation of bound FDP that occurred as soon as the enzyme was converted to the K form. The time constant of the apparatus was 1 ms. The figure reproduces the outline of the oscilloscope trace, and the smooth curve represents the equation

$$y/y_\infty = 1 - \exp(-4.4t)$$

In these experiments, FDP was used rather than FTP to avoid any possibility of phosphorylation, and its concentration was kept constant by including it in both syringes. Mixing was followed by a slow fall in fluorescence with a rate constant of about $4.4 s^{-1}$. Since, in a control experiment (not shown), displacement of FDP from the Na form of the enzyme by the addition of excess ATP led to a rapid fall in fluorescence ($k = 110 s^{-1}$), the slow fall in Fig. 4*b* presumably reflects a slow change from Na form to K form.

Although in the conditions of Fig. 4*b*, a K^+ concentration of 1 mM should have been sufficient to displace FDP almost completely (see Fig. 2), the rate of change of fluorescence was increased about fivefold when the K^+ concentration was increased from 1 mM to 5 mM. The amplitude of the change was unaffected. The increase in rate implies that binding of K^+ ions to a low affinity site accelerates the change in conformation, and suggests that the sequence of events is



where E_1 , the form of the enzyme with a high affinity for nucleotide, has a low affinity for K^+ . It is economical to suppose that E_2K in this equation is identical with ' $E_2(K)$ ', the form of the enzyme with occluded K^+ , in the scheme described above. The remarkably slow rates seen in the experiments like that of Fig.

4*a* are certainly compatible with the longevity of the 'occluded form' postulated by Post, Hegyvary and Kume¹³ at low nucleotide concentrations. If this identification is correct, it follows that the occluded form can be reached not only by K^+ -induced hydrolysis of phosphoenzyme, but also by conversion from a form of the enzyme that binds K^+ at low affinity (?intracellular) sites.

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General anaesthetics can selectively perturb lipid bilayer membranes

THE circumstantial evidence that anaesthetics act primarily by increasing the fluidity of membranes is quite strong. The gaseous, volatile, barbiturate, steroid and alcohol anaesthetics have all been shown to fluidise phosphatidylcholine-cholesterol lipid bilayers and the demonstration has also been made for some biological membranes¹⁻⁴. Furthermore, a number of lipophilic substances, such as the higher alkanols, do not fluidise membranes and are not anaesthetics⁵. In some cases, a correlation between nerve-blocking potency and the action of anaesthetics in perturbing lipid bilayers has been observed⁶. Moreover, pressure counteracts the fluidising effects of anaesthetics just as it antagonises general anaesthesia *in vivo*⁶⁻⁹. The overall success of the fluidised lipid hypothesis tends to be its major drawback, for if anaesthetics fluidise membranes indiscriminantly then the hypothesis fails to provide a unique mechanism for their selective depression of neuronal function. We show here that lipid composition may modulate the ability of an anaesthetic to fluidise membranes more than has been generally supposed.

The effect of anaesthetics on membrane fluidity has been studied by intercalating fatty acids, or phosphatidylcholine (PC), labelled with a nitroxide reporter group into lipid bilayer membranes of various compositions. The anaesthetics studied showed wide variations in their ability to fluidise phospholipid bilayers. Results and experimental conditions are outlined in Table 1. All anaesthetics studied fluidised PC bilayers low in phosphatidic acid (PA) and high in cholesterol (Chol) (4%PA: 33%Chol) as evidenced by the consistent decrease in order parameter. At one extreme, halothane and urethane fluidised all the membranes studied, whereas at the other, pentobarbital and alphaxalone fluidised only those with 4%PA: 33%Chol.

Increasing cholesterol content conferred fluidising ability on all anaesthetics, but increasing the negatively charged PA tended to confer an ordering ability in some cases. A more detailed examination of the effects of lipid composition was made with pentobarbital and octanol (Table 2). The effect of pentobarbital switched from fluidising to ordering when, in the 4%PA : 33% Chol membranes, either the cholesterol content was lowered to 5–10% or PA was increased from 4 to 10%. Octanol always fluidised.

Our results in 4%PA : 33%Chol membranes are consistent with previous studies which have been carried out primarily in membranes of this cholesterol content, which is typical of nerve^{2–4}. There are two isolated reports in the literature that halothane¹¹ and pentobarbital¹² order phospholipid membranes, as do local anaesthetics¹³.

Spin-label¹³ and deuterium magnetic resonance¹⁴ studies suggest that the acyl groups of phospholipids are tilted near the head group region. General anaesthetics might reduce this tilt leading to a decrease in packing density as has been reported for tetracaine¹³. If so, the ordering effect should be weaker deeper in the bilayer and preliminary results with PC labelled with 8-doxylstearic acid at the β position are consistent with this explanation. In this context it is interesting to note that the three anaesthetics which increase anisotropy in the 4% PA bilayer all have structures which include rigid rings. A complete physical explanation of our results must await more detailed study, however.

The most important pharmacological aspect of this work concerns the specificity of action of anaesthetics. Although they are regarded conventionally as nonspecific drugs, many membrane processes are unaltered at anaesthetic doses. Thus the Na⁺/K⁺ ATPase of red blood cells¹⁵ and synaptosomes¹⁶ is unaffected at high, almost lytic, doses. This specificity might, *a priori*, reside in the primary perturbation of the lipid as well as in the secondary reaction of a given membrane protein to that lipid perturbation. Our work shows that the primary perturbation can no longer be thought of in terms of the lipid solubility of an anaesthetic alone; it is necessary to introduce the concept of fluidising efficacy, which we define as the rate of change of membrane fluidity with the concentration of anaesthetic in the membrane. Defined thus, fluidising efficacy

Table 2 The change in order parameter caused by anaesthetics in PC lipid bilayer membranes containing varying proportions of PA and Chol

Anaesthetic	PA	Cholesterol				
		0	5%	10%	20%	33%
Pentobarbital 16 mM	4%	+0.02	+0.02	0	−0.02	−0.03
	10%	—	+0.05	+0.04	+0.03	+0.05
	20%	+0.02	+0.09	+0.05	+0.02	+0.03
Octanol 46 mM	4%	−0.02	—	—	—	−0.08
	10%	−0.05	−0.02	−0.03	−0.08	−0.04
	20%	−0.06	—	−0.03	−0.02	−0.03

need not be independent of anaesthetic concentration: thus Rosenberg reported unequivocal biphasic effects of halothane at physiological concentrations in palmitoyllauroyllecithin bilayers¹¹. Most studies so far, however, have shown linear effects^{2,3} or only weak nonlinear ones⁴. Three of the anaesthetics we examined could both order or fluidise bilayers, depending on their lipid composition, and thus indubitably exhibited both negative and positive fluidising efficacy; whereas for halothane, octanol and urethane, the fluidising efficacy was positive in all the membranes studied. For the latter anaesthetics we are unable to tell if the magnitude of the efficacy varies from membrane to membrane because the membrane solubilities are unknown. Our conclusions are thus based on the anaesthetics where a change in sign of the order parameter was observed.

To be consistent with the fluidised lipid hypothesis, anaesthetics should exhibit a positive fluidising efficacy at their site of action. In agreement with this, all six fluidised 4%PA : 33%Chol bilayers and pressure reversal of anaesthesia *in vivo* has been demonstrated for halothane^{8,9}, alphaxalone (as the clinical mixture althesin, which includes one third alphadolone acetate, a steroid with half the potency of alphaxalone)⁹, pentobarbital¹⁷, urethane¹⁸ and ketamine (M. Wilson and K. W. M., unpublished). Long chain alcohols, which are not anaesthetics and do not fluidise membranes^{3,19}, might have lower efficacy than the short chain alcohols, or might simply have a lower membrane solubility. In the latter case, if the efficacy is about normal they might act additively with other anaesthetics as has been observed for some fluorinated hydrocarbons²⁰. In all cases the magnitude of the fluidising efficacy can only be evaluated if the membrane partition coefficient is known, which is not often the case.

A corollary of the above argument is that only membrane regions where anaesthetics exhibit positive fluidising efficacy offer putative sites of action for anaesthetics. Thus, those anaesthetics, such as pentobarbital, which exhibit a positive fluidising efficacy in a restricted range of compositions might be useful for defining the nature of such sites. Our studies suggest they must have greater than 10% cholesterol and a charge density between 0.12 and 0.46 per phospholipid. This is consistent with the known compositions of neurones (30–50%Chol; 12–20% phospholipids bearing a single negative charge)²¹, although our phospholipids are not typical of the variety found in biological membranes. Two factors could limit the usefulness of such an approach. First, the presence of protein in a lipid bilayer will probably exert an additional influence on the fluidising efficacy of anaesthetics—a single report that halothane orders rat brain synaptic membrane is cautionary¹¹. Second, the lipids in biological membranes are heterogeneously distributed both across and in the plane of the membrane^{22,23}.

Large negative efficacies might also result in pharmacological effects²⁴; indeed lipid ordering has been suggested as the cause of the hyperbaric convulsions exhibited by mammals at pressures in excess of 70 atm (ref. 25). We have shown that merely increasing the PA content from 4 to 10% in a 33% cholesterol bilayer changes the fluidising efficacy of pentobarbital from plus to minus. It remains to be seen how far the delicate balance

Table 1 The change in order parameter, ΔS , measured by 5-doxylstearic acid in lipid bilayer membranes exposed to anaesthetic agents

Anaesthetic	Concentration	Bilayer composition, balance PC		
		4%PA	20%PA	4%PA:33% Chol
Halothane	11 mM	−0.03	−0.03	−0.06
Urethane	90 mM	−0.08	−0.03	−0.03
n-Octanol	46 mM	−0.02	−0.06	−0.08
Ketamine	25 mM	+0.03	0	−0.03
Alphaxalone	18 mM	+0.02	+0.03	−0.02
Pentobarbital	16 mM	+0.02	+0.02	−0.03

PC, Egg yolk phosphatidylcholine; PA, egg yolk phosphatidic acid; Chol, cholesterol; halothane is CF₃CHClBr; urethane is ethylcarbamate; ketamine is 2-(methylamino)-2-(2-chlorophenyl) cyclohexanone and was a gift of Parke-Davis; alphaxalone is 3 α -hydroxy-5 α -pregnane-11,20-dione and was a gift of Glaxo. Lipids and spin labels in organic solvents were dried down together in pear-shaped flasks. Involatile anaesthetics were added in organic solvents before drying down; volatile agents were added later after the lipids had been dispersed in solution buffered at pH 7.0 by vortexing, and their concentration was checked by gas chromatography. Final lipid concentration was about 20–30 mg ml^{−1}, and the spin labels constituted about 1 mol % of the lipids. Anaesthetics were equilibrated with the bilayers up to 24 h before being sealed in 1-mm glass capillaries and equilibrated at 25 $\pm$ 0.5 °C in the cavity of either a Varian E-9 or E-109 electron spin resonance spectrometer operating at 9.5 GHz. Order parameters, ΔS , were calculated from spectra according to the method of Hubbell and McConnell¹⁰. A decrease in ΔS indicates a less anisotropic distribution of the label and a more fluid membrane. Changes in ΔS less than 0.01 were not considered significant. High doses of anaesthetics were used to obtain large changes in ΔS , which is a linear function of anaesthetic concentration within experimental error^{23,25}.

between anaesthetic and convulsant activities seen with many barbiturates can be explained in this manner.

Our work has been concerned exclusively with the primary anaesthetic-induced lipid perturbation. The sensitivity of membrane proteins to such perturbations might also vary.

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Restriction map of 5S RNA genes of *Drosophila melanogaster*

ALTHOUGH certain segments of the primary sequence and secondary structure of 5S RNA seem to be strikingly similar in prokaryotes and eukaryotes¹, the genetic organisation of the 5S RNA genes themselves exhibits remarkable variability. In *E. coli*, several lines of evidence indicate that the multiple copies of the 5S, 16S and 23S rRNA genes are organised into transcription units containing one gene each plus a sequence coding either for tRNA^{1LE} or tRNA^{G1U} such that they are cotranscribed in the order 16S-4S-23S-5S RNA (refs 2 and 3). In eukaryotes, the 5S RNA genes may be clustered at a single site as in humans^{4,5} or widely distributed on many chromosomes as in the case of *Xenopus laevis*, where the 5S genes are found on the telomeres of most, if not all, of the 18 chromosomes⁶. Among the eukaryotes, there is no example in which the 5S genes are tightly linked to the 18S and 28S rRNA genes. Although there has been a large number of papers reporting the cytogenetic localisation of the 5S RNA genes in various organisms, only in the case of *Xenopus laevis* are there data concerning the physical organisation of these genes⁷. Even in this instance, however, the structure is only known for a few 5S gene repeat lengths, and the long range order that might be imposed on an entire 5S DNA cluster within the chromosome remains obscure. Whereas long range order in certain satellite sequences has been described^{8,9}, similar higher order organisation has not as yet been revealed for transcribed redundant genes.

In the fruit fly, *Drosophila melanogaster*, there are approxi-

mately 160 5S RNA genes (5S DNA) per haploid genome^{10,11}. Both genetic¹¹ and *in situ*¹² hybridisation data indicate that these genes are clustered in the euchromatic portion of chromosome 2 at the cytogenetic locus 56EF. Utilising a series of genetic deficiencies, we have shown that it is possible to split 160 tandemly repeated 5S RNA genes into two functional clusters, each containing about 80 genes¹¹. The results of experiments with restriction endonucleases reported here have allowed us to construct a high resolution restriction map depicting the physical organisation of these genes. The physical structure of the 5S DNA is similar to their genetic organisation in that the 160 genes are organised as two distinct clusters of 80 genes each.

On treatment of wild-type *D. melanogaster* DNA with *EcoRI* restriction endonuclease, the fragments are separated by electrophoresis on 1% Agarose slab gels and transferred to a cellulose nitrate filter according to the method of Southern¹³. The filter-bound fragments were hybridised with ¹²⁵I-labelled 5S RNA and finally visualised by autoradiography. As seen in Fig. 1, *D. melanogaster* 5S RNA hybridises to a single size class of 23 kb (kilobases or kilobase pairs) in length. Two independently isolated mutants (*min*) deficient for 50% of the wild-

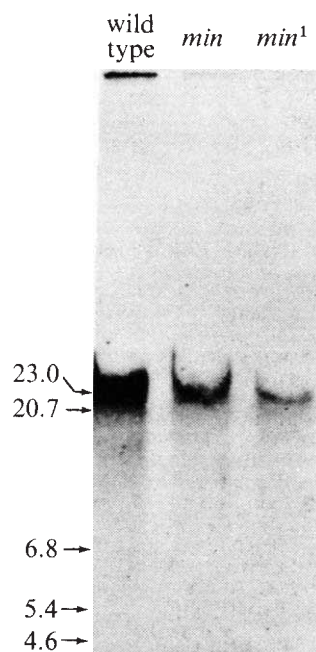


Fig. 1 Electrophoresis of *EcoRI* 5S DNA restriction fragments of wild-type and various *min* DNAs. *min* is a spontaneously derived mutant¹¹ and *min*¹ was obtained by triethylmalamine mutagenesis (Procunier and Tartof, unpublished). Each mutant contains half (0.003%) the number of wild-type 5S RNA genes. Approximately 10 µg of each DNA was digested with *EcoRI* in 0.05 ml of 50 mM Tris, pH 7.2, 50 mM NaCl, 10 mM MgCl₂ and 1 mM dithiothreitol at 37 °C for 2 h. A sufficient amount of enzyme was used to ensure complete digestion. The reaction was terminated by the addition of EDTA to 50 mM and NaCl to 0.20 M. The DNA was precipitated with 2 volumes of ethanol at -20 °C, collected by centrifugation and dissolved in E buffer (40 mM Tris, pH 7.2, 20 mM sodium acetate and 1 mM EDTA) containing 20% sucrose. Electrophoresis was carried out in 1% Agarose slab gels in E buffer for 4 h at 110 V. Following electrophoresis, the DNA fragments were transferred to a nitrocellulose filter according to the method of Southern¹³ and then hybridised to ¹²⁵I-labelled 5S RNA (5 × 10⁷ c.p.m. µg⁻¹). The hybridisation reaction was carried out for 20 h at 60 °C by placing the filter in 10 ml of 2 × SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) with 0.20 µg of labelled 5S RNA and 400 times this amount of unlabelled 18S and 28S RNA. The filter was washed with 2 × SSC and treated with RNase (20 µg ml⁻¹) in 2 × SSC for 1 h at 37 °C. After another washing, the filter was dried at 60 °C and exposed to X-ray film (Ilford) for 13 d. *EcoRI* restriction fragments of phage λ DNA served as size standards (20.7, 6.8, 5.4 and 4.6 kb)¹⁶. From these, the size of the 5S DNA fragment was determined to be 23 kb.

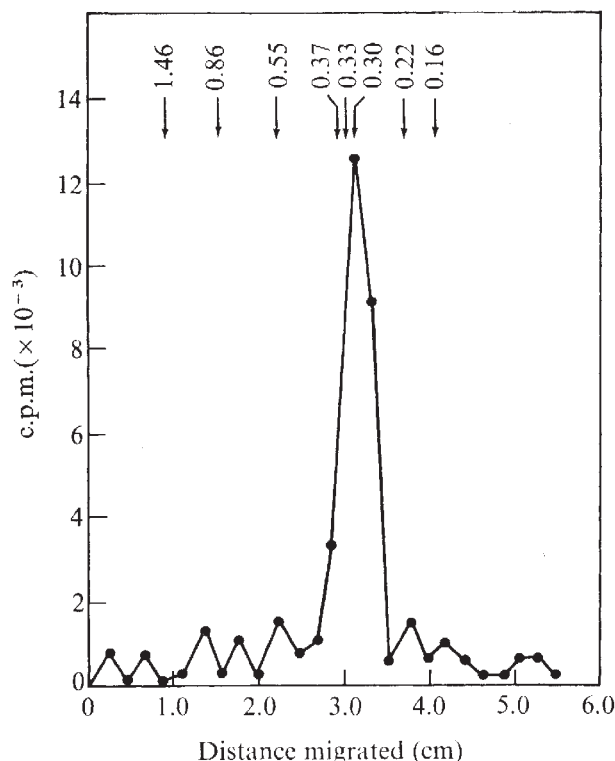


Fig. 2 Electrophoresis of *Hae*III 5S DNA restriction fragments. 400 μ g of wild-type DNA were completely digested with purified *Hae*III at 37 °C in 6 mM Tris, pH 7.4, 6 mM NaCl, 6.6 mM $MgCl_2$ and 3 mM dithiothreitol. The enzyme was provided by Dr Jesse Summers of this Institute and purified according to Middleton *et al.*¹⁷. After digestion the reaction was terminated, DNA fragments precipitated and dissolved in E buffer according to the protocol of Fig. 1. Electrophoretic separation of the fragments was accomplished in 6% acrylamide—0.1% diacrylate gels (9.2 cm long, 0.45 cm diameter) in E buffer at 5 mA per gel for 1.5 h. The gel was frozen and sliced into 2-mm sections and each section dissolved in 50 μ l of 1.5 N NaOH for 10 min at 70 °C. The mixture was neutralised with HCl and brought to a final concentration of 0.60 M NaCl, 0.20 M Tris and 0.02 M EDTA. The denatured DNA fragments were then covalently bound to phosphoimidazol-cellulose filters according to Saxinger *et al.*¹⁵. The 5S ^{125}I -hybridisation reaction and subsequent RNase digestion was done as described previously (Fig. 1) and the filters dried and counted. Background values (identically treated filters but containing no DNA) were subtracted from the experimental values. *Hae*III restriction fragments of SV40 DNA provided the molecular weight standards in kilobases¹⁸.

type number of 5S genes were similarly examined. The DNA from each of these also contains a 23-kb 5S DNA segment bounded by RI sites in spite of the fact that these mutants are deficient for half the wild-type number of these genes. This indicates there is more than one such 23-kb piece of 5S DNA per haploid genome.

To determine the number of *Eco*RI site bounded 5S DNA clusters, the precise size of the 5S repeating unit was obtained as follows. An *Hae*III cleavage point of 5S DNA can be predicted from the known *Drosophila* 5S RNA sequence¹⁴. The *Hae*III site



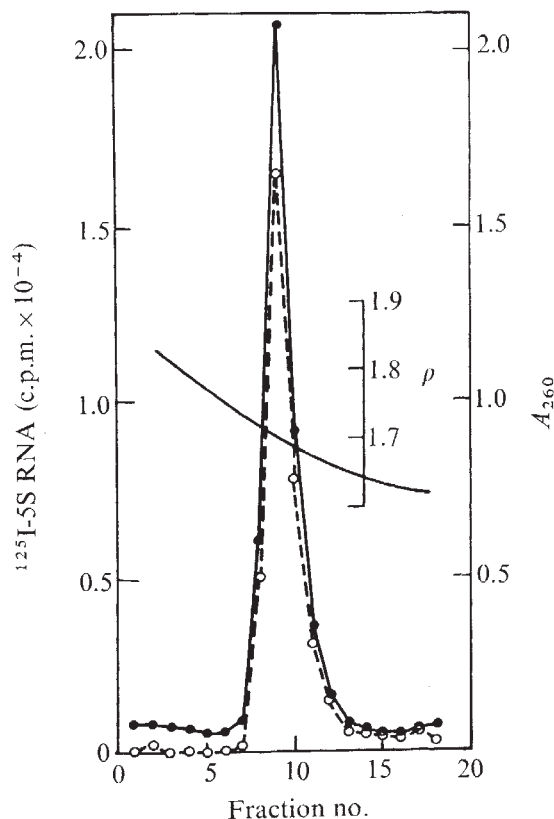
is between base pairs 116 and 119 of the 120 base pair 5S gene. The Southern technique which uses nitrocellulose filters however, fails to detect DNA fragments smaller than 500 base pairs¹³. Since it was quite possible that the 5S gene repeat size was smaller, we looked for a way round this difficulty. This was achieved by first separating the products of *Hae*III digestion by electrophoresis on a 6% polyacrylamide gel, hydrolysing the gel slices and denaturing the DNA fragments with alkali and then covalently binding the DNA fragments to a carbonyldi-

imidazole-derivatised phosphocellulose filter according to the procedure of Saxinger *et al.*¹⁵. The DNA-bound filters were then hybridised with ^{125}I -labelled 5S RNA. The results from this experiment are shown in Fig. 2, where a single *Hae*III fragment 290 base pairs long is found to hybridise to 5S RNA.

The results of the RI and *Hae*III restriction experiments suggest the following structure for the 5S DNA of *D. melanogaster*. 160 5S RNA genes with a repeat length of 290 base pairs would require 46.4 kb. The RI cuts embrace a cluster of 5S RNA genes 23 kb long. Therefore, we conclude that there are two RI site-bounded clusters, each containing 80 tandemly arranged 5S RNA genes. Each of these genes in turn consists of a 120 base pair structural gene and a 170 base-pair spacer. It is also known that 5S RNA is 57% GC, yet the genes band in a CsCl gradient in a position indicating that the average repeating unit has a base composition similar to the bulk DNA which is 40% GC (Fig. 3). So, we reason that the spacer is approximately 72% AT. A summary of the physical and genetic maps of *Drosophila* 5S DNA is given in Fig. 4.

It is possible that there could be a second *Hae*III site in the spacer region which would produce a second fragment that we would not detect in our experiments because the first *Hae*III site occurs three bases from the end of the 5S structural gene leaving an insufficient 5S DNA tag to probe for. The size of the *Hae*III fragments (290 base pairs) multiplied by the number of genes (160) however, fits almost exactly the dimension required to accommodate two RI site-bounded clusters (46.5 kb com-

Fig. 3 Buoyant density of 5S DNA. 100 μ g of wild-type DNA were mixed with CsCl such that the final density was 1.700 $g\ cm^{-3}$ in a volume of 7 ml. The solution was centrifuged for 50 h at 50,000 r.p.m. in a Beckman type 65 rotor at 20 °C. Fractions were collected and the absorption at 260 Å and buoyant density (ρ) measured. Each fraction was then denatured with alkali, neutralised and finally immobilised on a nitrocellulose filter¹⁹. The filters were then hybridised for 20 h at 60 °C in 3 ml of $2 \times$ SSC containing 10^6 c.p.m. of ^{125}I -labelled 5S RNA (5×10^7 c.p.m. μg^{-1}) and 100 μ g unlabelled 18S and 28S RNA. At the end of the incubation time, the filters were washed with $2 \times$ SSC, treated with RNase (20 $\mu g\ ml^{-1}$) for 60 min at 37 °C, washed again with $2 \times$ SSC and then dried and counted. $\circ$, ^{125}I -5S RNA; $\bullet$, A_{260} ; —, ρ .



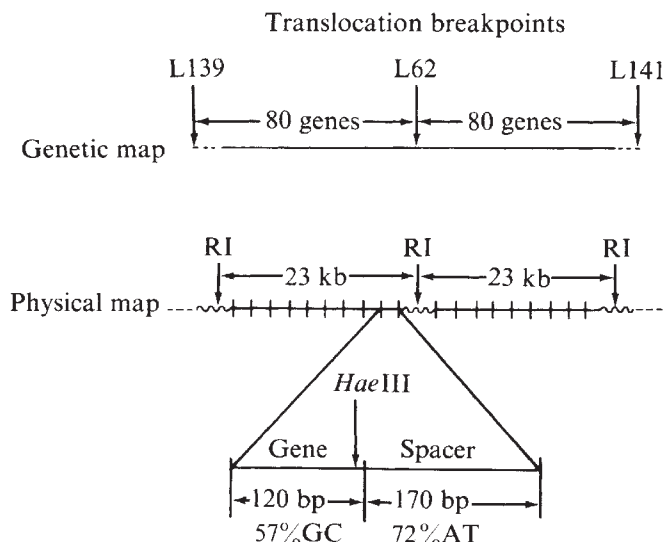


Fig. 4 A diagrammatic representation of the genetic and physical maps of the *Drosophila* 5S genes. From genetic evidence, it is known that the 5S DNA can be split into two functional clusters each of which contain approximately the same number of genes¹¹. The designation and position of the translocation breakpoints which result in splitting the chromosome in the 5S DNA region are indicated. The physical map agrees with the genetic map in that there are two 5S DNA clusters of approximately equal size and bounded at the ends by *EcoRI* sites. The precise number of *EcoRI* sites and size of the DNA segment between the two 5S DNA clusters is unknown. The position of the *HaeIII* site and the size and GC composition of structural gene and spacer are indicated.

pared with 46 kb), and this suggests to us that there is probably no other *HaeIII* site in the spacer.

Two interesting aspects of the 5S DNA restriction map deserve special comment. First, we do not know either the quantity or the quality of the DNA segment inserted between the two 5S DNA clusters. The fact that one translocation breakpoint occurs between the two 5S DNA blocks (Fig. 4) suggests that the amount of DNA in this region must be appreciable. Second, the restriction map does not allow assignment of the relative order of the two tandemly arranged 5S DNA clusters with respect to each other. These clusters may be organised as direct serial repeats (a-b-a-b) or as reverse repeats (a-b-b-a). Unequal exchange between two direct serial repeats would be expected to produce genotypes containing variations in the number of RI site-bounded 5S DNA clusters. Indeed, one might expect the number of such clusters to increase enormously in the course of evolutionary time. Although our sample size is small, we have not observed such instability¹¹. From such genetic considerations, we argue that a reverse repeat order of the two 5S DNA clusters would stabilise and preserve their structure in the chromosome. In addition, a palindrome arrangement would offer, on assuming the cruciform configuration, a means of maintaining homogeneity among both clusters through mismatch repair. We cannot but wonder if a similar arrangement might be imposed on other tandemly reiterated gene clusters.

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Two proteins function in the regulation of photosynthetic CO₂ assimilation in chloroplasts

THERE is increasing evidence that products of the light reactions of photosynthesis govern the activity of enzymes involved in CO₂ assimilation by chloroplasts¹⁻¹⁵. Of these products, reductants formed photochemically seem to be of particular importance. Such reductants include the reduced form of ferredoxin^{2,6,10}, a strongly electronegative chloroplast iron-sulphur protein ($E'_0 = -0.42$ V) that activates the two key chloroplast enzymes fructose 1,6-bis-phosphatase and sedoheptulose 1,7-bis-phosphatase. Activation of both of these enzymes requires in addition to reduced ferredoxin a 'protein factor' that is indigenous to chloroplasts. In efforts to elucidate the nature of the ferredoxin-linked enzyme activation, we have separated the protein factor into two components¹⁶: (1) a partly purified protein, provisionally named "assimilation regulatory protein a" (ARP_a) and (2) a highly purified chromophore-free protein called "assimilation regulatory protein b" (ARP_b). Only the latter was required for activation when reduced ferredoxin was replaced by the non-physiological sulphhydryl reagent dithiothreitol^{6,10}.

The protein factor was resolved into the ARP_a and ARP_b components by following the reported purification procedure (fractionation by acid pH, acetone, ammonium sulphate, and Sephadex G-100 gel filtration)⁶ that was modified to provide improved resolution in the gel filtration step.

Table 1 Requirement for ARP_a and ARP_b for activation of chloroplast fructose 1,6-bis-phosphatase by reduced ferredoxin

	nmol P _i released per min
Control	0
ARP _a	14
ARP _b	0
ARP _a plus ARP _b	73

The reaction was carried out in Warburg vessels containing (in the sidearm) 6 μmol of sodium fructose 1,6-bis-phosphate, and (in the main compartment) 135 μg of fructose 1,6-bis-phosphatase; spinach chloroplast fragments, P_iS₁, equivalent to 0.1 mg chlorophyll⁶; 0.12 mg of spinach ferredoxin, and the following (μmol): Tris-HCl buffer, pH 8.0, 100; neutralised reduced glutathione, 5; sodium ascorbate, 10; 2,6-dichlorophenolindophenol, 0.1; MgCl₂, 1.85 μg of ARP_a and 108 μg of ARP_b, were added as indicated. Vessels were equilibrated with nitrogen for 6 min and were then incubated for 5 min in the light. The reaction was started by adding fructose 1,6-bis-phosphate from the sidearm and was continued for 30 min under illumination. Light intensity, was 20,000 lx and temperature was 20 °C. The reaction was stopped by adding 0.5 ml of 10% trichloroacetic acid and (after removing the precipitate by centrifugation) P_i was estimated by a modified Fiske-SubbaRow procedure⁶.

¹ Fox, G. E., and Woese, C. R., *Nature*, **256**, 505-507 (1975).

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Although ARP_a and ARP_b were not completely separated, even after improvement in the gel filtration step, fractions of the two components were sufficiently resolved to establish their separate identities. ARP_b was then further purified by DEAE-cellulose chromatography and a second Sephadex G-100 gel filtration step. Details of the additional purification procedure will be published elsewhere.

Ultracentrifugation of purified ARP_b (single absorption maximum at 280 nm) showed one component with an approximate molecular weight of 20,000 but polyacrylamide gel electrophoresis yielded two components, both of which showed ARP_b activity. It seems therefore that ARP_b can exist in more than one form.

Table 1 demonstrates that both ARP_a and ARP_b were required for activation of fructose 1,6-bis-phosphatase by reduced ferredoxin. By contrast (Table 2), only ARP_b was required for activation of the enzyme when reduced ferredoxin was replaced by dithiothreitol. Similarly, the dithiothreitol-induced activation of the sedoheptulose 1,7-bis-phosphatase activity that occurs when the chloroplast fructose 1,6-bis-phosphatase is converted from its dimer to its monomer form when the pH is increased from 5.5 to 8.5 (ref. 17) required only ARP_b.

In addition to its activation of the fructose 1,6-bis-phosphatase and sedoheptulose 1,7-bis-phosphatase enzymes of chloroplasts, ARP_b was found to activate the regulatory form of NADP-glyceraldehyde phosphate dehydrogenase^{14,18} in the presence of dithiothreitol (Table 3). Neither the NAD-linked activity of the regulatory form of the enzyme nor the NAD(P)-linked activity of its non-regulatory counterparts was affected by ARP_b. In the presence of dithiothreitol, ARP_b also activated one of the two forms of phosphoribulokinase that we have found in chloroplasts (Table 3). In neither case could other sulphhydryl reagents replace dithiothreitol.

In summary, the evidence indicates that ARP_a and ARP_b, two newly discovered chloroplast proteins, link the strong reducing power of photochemically reduced ferredoxin to the regulation of certain enzymes of photosynthetic CO₂ assimilation. We believe that the ferredoxin-linked system constitutes a specific mechanism of enzyme regulation that operates in addition to the other light-actuated systems of chloroplasts. Of the latter group, those mechanisms involving enzyme effectors (such as reduced NADP) would govern

Table 3 Effect of preincubation with ARP_b and dithiothreitol on chloroplast NADP-glyceraldehyde phosphate dehydrogenase and phosphoribulokinase

	$\Delta A_{340 \text{ nm}}$ per min	
	Preincubation with	
	dithiothreitol	ARP _b plus dithiothreitol
NADP-glyceraldehyde phosphate dehydrogenase	0.30	1.14
Phosphoribulokinase	0.04	0.37

To assay NADP-glyceraldehyde phosphate dehydrogenase, 90 μg of the regulatory form of the enzyme¹⁴ was preincubated for 3 min in a solution containing (in a volume of 0.1 ml) 10 μmol of Tricine-NaOH buffer, pH 8.4; 1 μmol of dithiothreitol, and, as indicated, 9 μg of ARP_b. After preincubation, the mixture was injected into the assay mixture that contained, in a volume of 0.9 ml, 3 μg of 3-phosphoglycerate phosphokinase and the following (μmol): Tricine-NaOH buffer, pH 8.4, 40; dithiothreitol, 1.5; MgCl₂, 10; ATP, 5; 3-phosphoglycerate, 5; NADPH, 0.12. Enzyme activity was measured by following the change in absorbance at 340 nm. The temperature was 22 °C. To assay phosphoribulokinase²⁴ (the form present in the purified fraction containing the regulatory form of NADP-glyceraldehyde phosphate dehydrogenase¹⁴), 90 μg of the enzyme was preincubated for 3 min in a solution containing (in a volume of 0.1 ml) 10 μmol of Tris-HCl buffer, pH 7.5; 1 μmol of dithiothreitol, and, as indicated, 9 μg of ARP_b. After preincubation, the mixture was injected into the assay solution that contained, in a volume of 0.9 ml, 10 μg of phosphoribuloisomerase; 60 μg of lactate dehydrogenase; 30 μg of pyruvate kinase, and the following (μmol): Tris-HCl buffer, pH 7.5, 100; reduced glutathione, 10; MgCl₂, 10; ribose-5-phosphate, 2; phosphoenolpyruvate, 0.5; ATP, 0.5; NADH, 0.12. Enzyme activity was measured by following the change in absorbance at 340 nm. The temperature was 22 °C.

the activity of selected regulatory enzymes, whereas other mechanisms (for example, those involving light-induced increases in the pH (refs 19, 20) or Mg²⁺ concentration^{21,23} of the stroma or soluble phase of chloroplasts) would provide the environment necessary for the optimal activity of enzymes of the reductive pentose phosphate cycle.

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Table 2 Activation of the fructose 1,6-bis-phosphatase and sedoheptulose 1,7-bis-phosphatase activities of chloroplast fructose 1,6-bis-phosphatase by ARP_b and dithiothreitol

Treatment	nmol of P _i released per min	
	Fructose 1,6-bis-phosphatase	Sedoheptulose 1,7-bis-phosphatase
Complete	168	131
Minus MgCl ₂	7	1
Minus ARP _b	14	58
Minus fructose		
1,6-bis-phosphatase	6	4
Minus dithiothreitol	11	4
Minus dithiothreitol, plus reduced glutathione	10	4
Minus dithiothreitol, plus β -mercaptoethanol	11	5

To assay fructose 1,6-bis-phosphatase activity, the complete system contained 8 μg of the dimer form of fructose 1,6-bis-phosphatase¹⁷, 9 μg of ARP_b, and the following (μmol): Tris-HCl buffer, pH 8.0, 100; MgCl₂, 1; dithiothreitol, 5; and fructose 1,6-bis-phosphate, 6. The final volume was 1.0 ml. To assay sedoheptulose 1,7-bis-phosphatase, the complete system contained 16 μg of the monomer form of fructose 1,6-bis-phosphatase (obtained from the enzyme dimer by increasing the pH from 5.5 to 8.5 (ref. 17); 9 μg of ARP_b, and the following (μmol): Tris-HCl buffer, pH 8.0, 100; MgCl₂, 10; dithiothreitol, 5; sedoheptulose 1,7-bis-phosphate, 1.5. The final volume was 1.0 ml. In each case, the reaction (at 25 °C) was started by the addition of enzyme and stopped by the addition of 4 ml of the mixture used for P_i analysis (see Table 1).

matters arising

Biotic extinctions by solar flares

REID *et al.*¹ have suggested a mechanism by which solar protons might catastrophically deplete atmospheric ozone during a reversal of the Earth's geomagnetic field, when its shielding effect is weakened. Organisms would thereby be exposed to a harsher ultraviolet environment, producing extinctions, such as those Hays² observed, closely correlated with geomagnetic reversals in deep-sea sediment cores. They further suggest that mass extinctions, such as those which took place at the close of the Cretaceous, may have thus occurred. Reid *et al.* assume that during a reversal the geomagnetic field effectively disappears for $\sim 1,000$ yr. They also assume that solar flares sufficiently intense to cause extinctions occur at intervals of $\sim 1,000$ yr or more. We propose to examine the validity of these assumptions by comparing them with geomagnetic reversals identified by Tarling and Mitchell³ for the past 75 Myr, and small scale (radiolarian) and large scale (dinosaurian) extinctions.

Hays² found four radiolarian cases of extinction (involving 6 species) during the past 2.5 Myr, in which time 10 geomagnetic reversals occurred³. According to the model proposed by Reid *et al.*, the expected number of extinction events (E) is related to the probability of occurrence of a strong solar flare during any year (P_s) and the number of successive years during which the magnetic field is effectively absent (T) as

$$E = RTP_s \quad (1)$$

where R is the number of reversals during the period considered. Solving for P_s , and substituting the values cited above:

$$P_s = E/RT = 4 \times 10^{-4} \text{ yr}^{-1} \quad (2)$$

The value for P_s is lower than assumed by Reid *et al.* Given their estimate of $P_s = 10^{-3} \text{ yr}^{-1}$, to satisfy equation (1), the magnetic field would have to disappear for 400 yr during a reversal, an interval shorter than the generally accepted value⁴. A reduction in P_s would not, in our view, impair the utility of the model proposed by Reid *et al.* in producing periods of small scale extinction.

It is, however, evident that the terminal

Cretaceous (dinosaurian) extinctions affected a much broader range of organisms^{1,5} than the radiolarian ones described by Hays. The extinction of the dinosaurs occurred within a short but undefined interval ~ 18 Myr after the end of a lengthy period of normal polarity^{6,7}. During these 18 Myr, eleven reversals took place⁶, but the diversity of terrestrial and marine reptiles remained constant up to the end of the Cretaceous⁸.

A solar flare powerful enough to produce such extinctions would, therefore, have been much more powerful than any directly considered by Reid *et al.* Because no extinction as severe as those in which the dinosaurs were eliminated have subsequently taken place (or $E = 1$), and at least 197 reversals have since occurred ($R = 197$; set $T = 10^3 \text{ yr}$), then the frequency of a hypothetical giant flare according to equation (2) is

$$P_s = (1.97 \times 10^6 \text{ yr})^{-1}$$

or ~ 1 per 200,000 yr. Solar proton events have been studied only during the past 15–20 yr (ref. 1), and there is little hard evidence for the existence of giant flares, although instabilities in solar activity are now receiving more attention than formerly⁹. We therefore concur that the supernova model, as proposed by Terry and Tucker¹⁰ and Ruderman¹¹ remains worthy of consideration. Although the coincidence of a reversal and the terminal Cretaceous extinctions would invalidate neither model, Keating¹² notes that this coincidence has not been demonstrated.

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CRUTZEN AND REID REPLY—We certainly agree with the interesting remarks of Béland and Russell¹. Our model was primarily intended to provide a tentative explanation for the apparent mysterious association between geomagnetic polarity reversals and small scale extinctions, as documented by Hays². The relationship between polarity reversals and mass extinctions, such as that at the close of the Cretaceous, is not well established, and our mechanism is only a possible candidate.

In terms of ionising radiation, the solar flares of August 1972 dissipated $\sim 6 \times 10^8 \text{ erg cm}^{-2}$ in the polar stratosphere. A similar flux of energy over the entire projected area of the Earth would be caused by the γ -ray 'pulse' ($\sim 10^{48} \text{ erg}$ (ref. 3)) from a supernova 1,000 light yr away, while a supernova 30 light yr away would create a flux 1,000 times larger. It is not likely that the Sun could produce such a colossal flare, but several supernovae may have occurred within a distance of 30 light yr during the lifetime of the Solar System⁴.

Although the shielding of ultraviolet light by NO_2 must be considered, any ozone depletion caused by such supernovae would seriously affect the biosphere⁵. However, other consequences of the vast amounts of nitrogen fixed in the atmosphere are worth considering. Assuming maximum efficiency of NO production, the supernova would fix $\sim 1,500$ Mt of nitrogen, which is almost 10 times the presently estimated annual global nitrogen fixation rate⁶. This could perturb ecological systems. Furthermore, the column density of NO_x could initially reach unhealthy concentrations of 10^{19} – 10^{20} molecules per cm^2 and although detailed calculations are necessary to confirm the idea, it may seem that there could be substantial production of NO_2 and even O_3 by the reaction $2\text{NO} + \text{O}_2 \rightarrow 2\text{NO}_2$. Given absorption cross sections of $\text{NO}_2 > 10^{-19} \text{ cm}^2$ at wave lengths $\lesssim 650 \text{ nm}$, severe perturbations in photosynthesis rates and in the radiation balance of the Earth may result.

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Glaciations and dense interstellar clouds

As a possible cause of an ice age on Earth, I have suggested the passage of the Solar System through a region of compression of interstellar matter (ISM) bordering a spiral arm of the Galaxy¹. Dennison and Mansfield criticise the model because it leads them to expect to find a dense cloud of ISM still very close to us; no such cloud is seen². Their criticism, however, ignores the structure of the Galaxy that provides the basis for my suggestion, and the justification for reviving the idea of a possible relation between ISM and ice ages.

The compression region is a shock region, that is, a 'traffic jam', in the ISM. According to the model, the most recent glaciation was associated with the Sun having been immersed in a cloud of ISM while it traversed such a region. On my interpretation, the Sun emerged from the region after that. Dennison and Mansfield adopt $\sim 20 \text{ km s}^{-1}$ for the relative speed of the Sun and cloud, and $\sim 10^4 \text{ yr}$ ago for the time of emergence.

Dennison and Mansfield take no account of the fact that the Sun and cloud were travelling through the region with mean speed $\sim 250 \text{ km s}^{-1}$ in their orbital motion round the Galaxy. So the place where the Sun emerged from the cloud is more than 10 times further off than in the reckoning of Dennison and Mansfield.

The material of the cloud concerned did indeed emerge from the compression region about the same time, but there are three essential points to recognise: (1) The region forms an oblique shock and, viewed as such, it produces a discontinuity in direction of flow of the ISM passing through it, but not of the stars. After emerging from the region, the cloud material and the Sun have relative motion very different from what they had in it. The traffic-jam is like congestion on an escalator—after people get off an escalator they disperse with relative speeds comparable to the speed of the escalator, not to the relative speed they had while on the escalator. (2) When the material emerges it is no longer compressed, since by definition the compression region is the region where it is compressed. Compression regions themselves are common places of observation, but how the compressed material evaporates out of them is not understood in detail. (3) There is no reason to suppose that clouds retain their individuality as the ISM evaporates from a compression region.

The answer to Dennison and Mansfield is that the dense cloud they expect is not where they infer, it is not dense, and it is not a cloud.

Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

For reasons I have stated briefly elsewhere^{1,3}, the end of the most recent glaciation could have been the very end of a period of glacial activity that did not even start until after the Sun had emerged from the cloud, and its luminosity had fallen back to normal after an interval of enhancement. Thus the time, $\sim 10^4 \text{ yr}$, is almost certainly an underestimate. In their closing paragraph Dennison and Mansfield go some way towards admitting this further general consideration and its implications. I should take issue with them over several other particular matters were this necessary. It seems, to me, however, that their objections vanish in the light of the foregoing general considerations, and that their discussion actually helps to show that there need be no embarrassing side effects in the model.

I was aware of the problem of a nearby cloud raised by Hoyle and Lyttleton in a paper⁴ I quoted in ref. 1, and recalled by Dennison and Mansfield, but ideas on the Galaxy and the kinematics of ISM have changed since 1939.

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¹ McCrea, W. H., *Nature*, 255, 607–609 (1975).

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DENNISON AND MANSFIELD REPLY—On encountering a spiral density wave, interstellar matter (ISM) suffers rapid and substantial changes in its flow field and density. Behind the shock, the gas gradually recovers over some millions of years¹. The actual shock region is extremely narrow, and thus the transit time of the stars and gas through it is negligible in comparison to a million years, the approximate duration of the last general ice age. Behind the shock, the ISM, or any cloud, could not undergo significant changes in velocity, den-

sity, or identity caused by spiral density wave in the $\sim 10^4 \text{ yr}$ since the last glaciation, or even in the $\sim 7 \times 10^4 \text{ yr}$ since the beginning of the last glaciation. To accomplish the rapid changes now proposed by McCrea², an unjustified assumption of a second abrupt change in the flow field of the ISM behind the initial shock would have to be made. The compression region fades gradually—it does not end abruptly like people leaving an escalator.

Before entering the compression region, the stars and ISM have a small velocity differential of $\sim 10 \text{ km s}^{-1}$, because they follow similar orbits. In entering the compression region, the ISM is severely perturbed, while the stars are largely unaffected. This produces velocity differentials between the stars and gas that are an order of magnitude larger than the $5\text{--}25 \text{ km s}^{-1}$ that McCrea uses. His low velocity differentials are appropriate for regions outside the compression zone. Since the 'traffic jam' applies to the ISM only, it is in the compression zone that there are large velocity differentials, and because the accretion rate in the model depends linearly on the cloud density and inversely as the cube of the velocity differential, the same fractional increase in solar luminosity would now require the frequent encountering of clouds with embarrassingly high densities, from $\sim 10^8$ to $\sim 10^{10}$ hydrogen molecules cm^{-3} .

Even if we accept the unorthodox picture that McCrea now uses, an excessive amount of energy is needed to dissipate these dense clouds on time scales of $\sim 10^4 \text{ yr}$. A typical cloud required by the original model has $\sim 10^6$ hydrogen molecules cm^{-3} and a diameter of $\sim 1 \text{ pc}$. Such dense clouds cool rapidly and cannot store compressional energy. It is easy to show that over a time of $\sim 10^4 \text{ yr}$ a power of $\sim 5 \times 10^4 L_{\odot}$ is required to overcome the gravitational binding of the cloud. McCrea's present model would require the dissipation of many such clouds throughout the disk of the Galaxy. This requires that on the average a power $\gtrsim$ total luminosity of the Galaxy must be constantly expended in dissipating dense clouds.

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¹ Woodward, P. R., *Astrophys. J.*, 195, 61–73 (1975).

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reviews

Provocative and rewarding genetics

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Genetics, Evolution and Man. By W. F. Bodmer and L. L. Cavalli-Sforza. Pp. xv+782. (Freeman: San Francisco, 1976.) \$13.95.

FIVE years ago, these authors gave us *The Genetics of Human Populations* (1971), a book addressed more to the specialist or advanced student than the neophyte. *Genetics, Evolution and Man* projects the notions set forth in this earlier effort on to a wider, less specialised readership. Their newer book expands the admirable features of its predecessor—excellent illustrations, conceptually and graphically; succinct abstracts at the beginning of each chapter, providing a sense of direction for the reader; the intercalation of frequent one- or two-line statements which capture the essence of the paragraphs which follow; problems which test and supplement one's understanding of each chapter; and a lucid, readily readable literary style. More important, perhaps, is its currency and the overall objectivity which Bodmer and Cavalli-Sforza bring to the task which they set for themselves. This does not imply that every human biologist will subscribe fully to their arguments on controversial issues—for example, the roles of heredity and environment in the observed differences among the races in tests of intelligence. Even where honest differences of opinion exist, however, it should be clear to all save the most committed that they make a serious appeal to reason rather than exhibit a preconception or prejudice.

This is unquestionably an important book, and everyone familiar with the subject matter must appreciate the wealth of information to which the uninitiated reader is exposed. It is provocative, but not provoking; it rewards without remonstrating. Each reader, indeed each reviewer, will undoubtedly be attracted to a different chapter. One of my favourites is the "Evolutionary Development of Modern Man". It attempts a synthesis of man's biological and social evolution as a human population geneticist might see it. The observations on which this synthesis is based are, of course, sensitive to substantial differences of interpretation both as to time and significance. Illustrative of such differences are the re-

ferences to the Jomon culture of Japan. Development of pottery by this culture is cited to be 9,000 yr ago, but *Japan; Its Land, People and Culture* (UNESCO 1958) suggests this event actually occurred about 14,000 yr ago; whereas the 'earliest Jomon' is given by Komatsu (*The Japanese People*, 1962) as about 6,400 yr ago. Apparently, Bodmer and Cavalli base their estimate on the carbon dating, carried out at the University of Michigan some years ago, of charred wood recovered from the Natsushima shell-mound in Kanagawa Prefecture. This specific example is not particularly important and quite possibly the figure given by Bodmer and Cavalli may be correct, but some of the 'facts' of their general argument will undoubtedly prove to be in error. This, however, is a much lesser moment than the synthesis and its respect for the divergent data sets which must, in some manner, be integrated. Their view does not disregard man's cultural evolution nor does it consciously serve the biological sciences. They acknowledge man's biological and cultural heritage; and seek

a quantitative appraisal of the contributions of these elements to his present diversity, not a capitulation on the part of one or the other disciplinary views.

If fault one must find, then this book is overly long. This stems, I believe, first, from a misdirected effort to make the book self-contained—that is, to provide the reader with every detail necessary to understand some of the subsequent arguments; and second, a failure to realise the frequency with which most students are exposed to the structure of DNA, the mechanics of mitosis, and elementary notions of statistics. This is not to suggest that reinforcement is not needed for some—possibly a large number—but a general application of repetition can just as readily lead to boredom as understanding. This is a small caveat, and doesn't seriously compromise the book's general excellence. □

William Schull is Director of the Center for Demographic and Population Genetics at the University of Texas Health Science Center, Houston, Texas.

Introduction to methods of geophysics

Geophysical Methods in Geology. (Methods in Geochemistry and Geophysics, Vol. 12.) By P. V. Sharma. Pp. xv+428. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) Dfl. 65; \$26.25.

HERE is a book that attempts to cover the techniques used in the whole field of geophysical research on the crust and lithosphere, industrial and academic; that is, a book that combines the contents of books such as Parasnis (1972), Bott (1971), York and Farquhar (1972) and Cox (ed. 1973). There are chapters on seismic methods (with sections on fundamentals of seismic wave propagation, seismic seismicity and seismotectonics, seismic prospecting, use of surface waves in crustal studies), gravity methods, magnetic methods (from Wenner spreads to magnetotellurics), radiometric methods (dating and prospecting), geothermal methods

and, finally, geophysics applied to global tectonics. The book is polymath, up-to-date and non-mathematical; SI units are used and a point in its favour is that most subsections end with a reference to a recent review article. It is a long book but not a verbose one.

Dr Sharma writes that the book developed from a course organised at the University of Copenhagen. "The course was aimed at providing a concise but fairly comprehensive introduction to the methods of geophysics within which the undergraduate students of geology and geophysics could locate their specific interests for further specialisation." He does not tell us in which year of their undergraduate career these students received this course. No man could have a lively research acquaintance with one half of the subjects touched on here so that the course (and the book) must have represented a con-

siderable piece of scholarship; but I feel that, in consequence, the book is at once too comprehensive and too facile to arouse the lively interests of an undergraduate reader. I shall continue to encourage the second- and third-year undergraduates whom I encounter to read Parasnis, Bott and Cox.

D. Matthews

Dr Matthews is a reader in the Department of Geodesy and Geophysics at the University of Cambridge, UK.

Planetary interaction

Interplanetary Encounters: Close-Range Gravitational Interactions. (Developments in Solar System and Space Science, 2.). By Ernst J. Öpik. Pp. vii+155. (Elsevier Scientific: Amsterdam, Oxford and New York, 1976.) \$26.95; Dfl.67.

THIS is an excellent book, a book that any scientist will read with awe, realising that very few of their number can, in the fullness of years, produce such a resumé of original work.

Ernst Öpik has been an astronomer of international renown since the early 1920s, his main interest centring on the Solar System. In this book he deals with the encounters between planets and between planets and stray bodies such as meteorites, asteroids and comets. These gravitational interactions sometimes lead to collisions—more often to changes in the orbital parameters of the two bodies and the possible ejection of one from the Solar System. Öpik has advanced from the restricted three-body problem to formulate a probability method reliant on two-body interactions over specific spheres of influence. This leads to a statistical celestial mechanics which culminates in the prediction of the cratering densities of Mars and Moon, dynamical lifetimes of cometary debris and other stray bodies, solutions to the Neptune-Pluto problem, stability of the Trojan asteroids, and many more phenomena.

The book summarises and refines a series of some 29 research papers written by the author between 1951 and 1972; it is beautifully produced, very clearly indexed and is essential reading for all Solar System astrophysicists.

David W. Hughes

David Hughes is a lecturer in the Department of Physics at the University of Sheffield, UK.

Quite recently, biological motivation has contributed new ideas to computer science and mathematics, while these disciplines have provided useful insights to biological development. North-Holland takes pleasure in detailing below three authoritative publications covering this new field.

Developmental Systems and Languages

by GABOR T. HERMAN and GRZEGORZ ROZENBERG.

With an introductory chapter by ARISTID LINDENMAYER, Subfaculty of Biology, University of Utrecht.

1975 xvi + 366 pages 10 tables 59 illus. over 200 lit. refs.
Price: US \$28.95 / Dfl. 75.00; ISBN 0-7204-2806-8

The application of the rigorous techniques of formal language theory to biologically motivated concepts gave rise to the theory of developmental systems and languages. This area has been actively investigated over the last few years, yielding many results of fundamental interest to both the formal language theorist, the biologist and the computer scientist.

Self-contained, this book provides an exhaustive and up-date survey of the field. After an introduction in which the biological motivation of the study is described, the book is divided into three main sections. Part I gives a formal presentation of the theory of *developmental languages* while Part II treats the theory of *developmental sequences*. Part III offers a less formal discussion of topics whose biological motivation is more apparent.

Automata, Languages, Development

At the crossroads of biology, mathematics and computer science

edited by ARISTID LINDENMAYER and GRZEGORZ ROZENBERG.

1976 viii + 529 pages Price: US \$46.00 / Dfl. 120.00; ISBN 0-7204-0474-6

A truly interdisciplinary exposition, this book represents the work of many distinguished scientists, brought together by their common interest in understanding the *algorithmic nature of developmental processes*. It includes both survey articles and specialized research papers covering recent trends in each of the following interrelated areas:

- mathematical and computer models for growth and development of multi-cellular organisms,
- the theory of L systems, and its relation to the formalization of multi-cellular development and regeneration,
- the theory of cellular automata and tessellation structures, and its relation to the concept of self-reproduction,
- the theory and applications of graph generating and related systems.

This book is based largely on the extension of material presented at a conference held at Noordwijkerhout, Holland, in April, 1975. Biologists, mathematicians and computer scientists will find this work a thoroughly representative and balanced review of the latest developments in an increasingly important interdisciplinary area.

Analysis and Control of Immobilized Enzyme Systems

edited by DANIEL THOMAS and JEAN-PIERRE KERNEVEZ.

1976 vii + 306 pages 22 tables 119 illus.
Price: US \$27.50 / Dfl. 70.00; ISBN 0-7204-0361-8

This volume contains the proceedings of an international symposium held in Paris, May 1975 which was devoted to analysis and regulation of immobilized enzyme systems. The aim of the symposium was to bring together researchers from different fields, viz., biochemistry, applied mathematics and computer sciences, with the main emphasis laid on experimental and theoretical analysis of immobilized enzyme systems as biological models. The identification of kinetic parameters as well as the control of enzyme systems ruled by partial differential equations, are presented.

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obituary

Walter Schottky, author of many significant contributions to the fields of electron and solid-state physics, died on March 4, 1976 at the age of 89. Born in Zürich, he studied under Max Planck at the University of Berlin, and subsequently held university appointments in Würzburg and Rostock. His first research interest lay in the field of electron physics, and what is now universally called the 'Schottky effect' (the increase in the thermionic emission of electrons from a solid resulting from an external electric field) dates from as far back as 1914.

Possibly as a result of the First World War, Schottky next turned his attention to the radio and in 1915 invented the screened-grid vacuum tube. He is also credited with the invention of the superheterodyne receiver. He studied the 'shot noise' which arises from the discrete nature of the charge carriers in a current of electrons, and in 1918 derived the well-known relationship between the magnitude of the noise and the strength of the current. For these three contributions he was awarded the Hughes Medal of the Royal Society in 1936.

After the war, Schottky's interests turned to thermodynamics and statistical mechanics. One result of this was his prediction of the 'Schottky specific heat anomaly'—the specific heat of a system possessing two closely spaced, discrete energy levels shows a maximum at a temperature comparable with the separation between the levels divided by Boltzmann's constant. His analysis

successfully explains specific heat behaviour which has been observed on numerous occasions by low-temperature physicists. His postulation of the existence of 'Schottky defects' in crystals (missing atoms without compensating atoms in interstitial positions) also dates from this period.

In 1927, Schottky became closely associated with the firm of Siemens, thereby continuing a well-established German tradition. From that time onwards he turned his attention to semiconductors, and in particular to the properties of metal-semiconductor contacts, and it is for this work that he is probably most widely known today. It had been known since the early work of Braun in 1874 that metal points in contact with certain solids, such as lead sulphide, show rectifying properties, and although these point-contact rectifiers were not understood, they nevertheless played a most important role as detectors in the early days of radio. In 1931 Schottky and his co-workers showed that in copper oxide rectifiers nearly all the potential drop occurred near the metal contact, thereby implying the existence of some sort of potential barrier. By this time quantum mechanics was firmly established, and Wilson and others tried to explain the rectifying action in terms of the quantum-mechanical tunnelling of electrons through the barrier, but it was soon recognised that this mechanism predicted the wrong direction of easy current flow. Schottky, and independently Mott, suggested that the rectify-

ing action resulted from the thermal excitation of electrons over the barrier, and showed that this model predicted the correct direction of rectification. Schottky supposed that the barrier region contained a space charge caused by a uniform density of charged impurities, so that the electric field increased as the metal was approached, whereas Mott assumed that the conditions of fabrication were such that the barrier region was devoid of impurities, so that the electric field was constant. We now know that Schottky's assumption is the more realistic one in practice, and in consequence the term 'Schottky barrier' is used almost universally to describe a metal-semiconductor contact. Schottky also derived an expression for the capacitance of the contact in terms of the density of charged impurities, and showed how measurements of the capacitance could be used to infer the impurity concentration. This technique is used very extensively throughout the semiconductor industry today.

Schottky was a very versatile physicist who combined a wide theoretical knowledge with a deep physical insight and the ability to look at problems from a practical point of view. He was always ready to apply his science to engineering problems, and it is perhaps appropriate that the term 'Schottky diode' is now firmly established in the vocabulary of electronic engineers who probably know little of his contributions to basic physics.

E. H. Rhoderick

The sudden death of **Professor Richard Foster Flint** of Yale University on June 6, 1976, deprived the world of one of the most eminent of its Quaternary geologists of any generation. Born in Chicago, Illinois, on March 1, 1902, the son of Professors Nott William and Edith Burnham (Foster) Flint of the University of Chicago, he obtained his B.S. in 1922 and his doctorate, *summa cum laude*, from that University in 1925. He joined the Yale University faculty as Instructor the same year and taught at Yale for the next 45 years. He retired in 1970 as Henry Barnard Davis Professor of Geology, the occasion being commemorated by a symposium in his honour and a resulting Festschrift (*The Late Cenozoic Ice Ages*) to which some of the world's leading researchers contributed.

Professor Flint's publications number over 150 research papers and a series of monographs including *Glacial Geology and the Pleistocene Epoch*

(1947), *Glacial and Pleistocene Geology* (1957), and *Quaternary and Glacial Geology* (1971). The last stands as the most up-to-date, comprehensive, and masterful overview of the subject in any language. He was also the co-author of a number of editions of such famous Yale texts as *Physical Geology* and *Introduction to Physical Geology*. Especially influential was his stimulating teaching that opened the exciting vistas he saw to generations of Yale graduate students and undergraduates—thousands of the latter benefited from his introductory course on the earth sciences. He also enhanced Yale's reputation by serving as associate-editor, co-editor, or member of the editorial board of a number of scientific journals, including *American Journal of Science*, *Quaternaria*, *Quaternary Research*, *Radiocarbon* and *Zeitschrift für Geomorphologie*. In addition he was Chairman of the National Research Council committees that compiled the

Glacial Map of North America and the Glacial Map of the United States.

Professor Flint once said that he did not expect great fame as a scientist, since he desired a life whose breadth would necessarily detract from single-minded scholarship. Yet he not only achieved the full life he sought, including a deep satisfaction in art and pottery, but contrary to his expectations, he also gained the world's acclaim as a scientist. He was a Fellow of the American Academy of Arts and Sciences, honorary member of many foreign geological societies and President of the 7th Congress of the International Quaternary Association.

Professor Flint leaves his devoted lifelong partner, Margaret C. H. Flint, a daughter, Anne Ogilvy, three grandchildren, one great grandchild, and a host of admirers, friends, and colleagues the world over.

A. L. Washburn

announcements

Appointment

Dr J. Ronayne to the Chair of History and Philosophy of Science at the University of New South Wales.

Professor Charles Antony Richard Hoare as Professor of Computation at the University of Oxford.

Dr Theodore Morris Sugden, Master of Trinity Hall, Cambridge, will be made President Elect of the Chemical Society in April 1977.

Professor Edward A. Smuckler as Chairman of the Department of Pathology, at the School of Medicine, UC-San Francisco.

Awards

The Paul Ehrlich awards for cell research have been given to **Professor John Gurdon**, of the Institute for Molecular Biology, and **Professor Torbjørn Caspersson**, of the Kungliga Karolinska Mediko-Kirurgiska Institutet, Stockholm.

The Royal Society Mullard Award for 1976 has been given to **Dr G. H. Hitchings**, recently retired from Burroughs Wellcome Co., for his work on chemotherapy, particularly the development of the drugs trimethoprim, allopurinol and azathioprine.

The Royal Society Esso Award for the Conservation of Energy has been made to **Mr. T. B. Jackson** of Honeywell Ltd.

The Ramsay Memorial Fellowships Trustees have awarded their General Fellowship to **Dr M. W. Evans** of the University College of Wales, Aberystwyth and the Netherlands Fellowship to **Dr M. R. Egmond** of the University of Oxford.

Meetings

September 22–24, **Systems and Models in Air and Water Pollution**, London (The Institute of Measurement and Control, 20 Peel Street, London W8 7PD, UK).

September 28–30, **Cellular Aspects of Neoplasia**, London (Dr Gisele M. Hodges, Department of Cellular Pathology, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK).

September 29, **Radioisotopes in Cardiology**, London (The General Secretary, British Institute of Radiology, 32 Welbeck Street, London W1M 7PG).

October 7, **Biology of the Pycnogonida**, London (The Executive Secretary, The Linnean Society of London, Burlington House, Piccadilly, London W1V 0LQ, UK).

Person to Person

Conoco Ltd announces the setting up of The Conoco Lecture on the Responsibility of Industry towards the Environment. The Conoco Lecture, which will become an annual event, will be presented to an invited audience in the spring of 1977 and will be submitted to the appropriate journal in the usual way. The Lecturer will have about four months to prepare his work and will receive a research award of £1,500. Nominations must be received by September 30, 1976.

Wanted, three- or four-bedroom flat or house, preferably furnished, in the West London area near to Ducane Rd. Will exchange for a three-bedroom flat in Turku, Finland from about October 25, 1976 to March 25, 1977. Replies (by September 20, please) to Docent Lauri J. Pelliniemi, M.D., c/o Shirley J. Green, Department of Histo-chemistry, Hammersmith Hospital, London W12.

Will send, for postage, small quantity of *Sophora chrysophylla* seed to persons interested in tropical highlands. Leguminous tree, frost tolerant, good timber, to 10 m high and 50 cm diam, Hawaii native. Perhaps of value in regions with climates similar to Quito, Ecuador. Apply to: W. Cook, PO Box 22364 Honolulu, HI. 96822.

Anyone going to Moscow who would like further information on the Moscow Seminars (see *Nature*, August 19) should contact: Joan Dale, Medical and Scientific Committee, 2 Froggnal Rise, London, NW3.

There will be no charge for this service. Send items (not more than 60 words) to Martin Goldman at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

October 14–19, **Warm Water Zooplankton**, Goa, India (Dr S. Z. Qasim, Director, National Institute of Oceanography, Dona Paula, PO Caranzalem, Panaji, Goa, India).

November 10, **Gerontology**, London (The Hon. Secretary, British Council

for Ageing, c/o The National Corporation for the Care of Old People, Nuffield Lodge, Regent's Park, London NW1 4RS).

September 5–9, 1977, **24th International Field Emission Symposium**, Oxford (Dr G. D. W. Smith, Dept of Metallurgy and Science of Materials Oxford University, Parks Road, Oxford, UK).

December 12–14, 1977, **Monitoring Hazardous Gases in the Working Environment**, London (Deadline for abstracts: January 31) (Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN, UK).

March 29–April 1, 1978, **Conference on Sub-Millimetre Waves**, Guildford, UK (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1 8QX, UK).

August 16–23, 1978, **Plant Pathology**, Munich (Congress Plant Pathology, Biologisches Bundesanstalt, Messeweg 11/12, D 3300 Braunschweig, FRG).

August 30–September 6, 1978, **Virology**, The Hague (Fourth International Congress for Virology, Netherlands Congress Centre, PO Box 9000, The Hague, The Netherlands).

Reports and Publications

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 281, No. 1302: New Tensor Spherical Harmonics, for Application to the Partial Differential Equations of Mathematical Physics. By R. W. James. Pp. 193–221. UK £1.45; Overseas £1.50. Vol. 281, No. 1303: Continental Displacement and Expansion of the Earth During the Mesozoic and Cenozoic. By H. G. Owen. Pp. 223–291. UK £4.10; Overseas £4.25. (London: The Royal Society, 1976.) [14]

The Zoological Society of London. Annual Report, 1975. Pp. 50. (London: The Zoological Society of London, 1976.) [24]

Malaysian Rubber Producers' Research Association. MRPRA Publications, 1938–1974: An Author Bibliography. Pp. 106. (Hertford: MRPRA, 1975.) £2. [54] UKAEA, Harwell, AERE-R 8267: Radioactive Fallout in Air and Rain—Results to the End of 1975. By R. S. Cambray, Miss E. M. R. Fisher, J. D. Eakins and D. H. Peirson. Pp. 49. (London: HMSO, 1976.) £1.50 net. [54]

Science Research Council. Daresbury 1975. Pp. 52. (Daresbury, Warrington: Librarian, Daresbury Laboratory, 1976.) [54]

Australian Journal of Ecology. Vol. 1, No. 1, January 1976. Edited by Derek Anderson. Pp. 1–66. Published quarterly. Annual subscription £15; \$A30 (Australia); \$52.50 (N. America); £18 (overseas). (Oxford and London: Blackwell Scientific Publications, 1976. Published for the Ecological Society of Australia.) [54]

Science Research Council: Engineering Board. Engineering Computing Requirements—Technical Group Report. (London: Science Research Council, State House, High Holborn, 1976.) *gratis*. [64]

The Gale's Book of Countryside Projects. Pp. 24. (Carrow, Norwich: Countryside Offers, Colman Foods, 1976.) 10p. [74]

Science and Technology in Islam. Pp. 48. (An exhibition at the Science Museum, London, 7 April–29 August, 1976.) (London: Science Museum, 1976.) [74]

A Challenge for Change in the Dental Services. (A Consultative Document prepared by a Working Party under the Chairmanship of Mr. Laurie Pavitt, M.P.) Pp. 36. (London: Literature Sales, The Labour Party, Transport House, Smith Square, 1976.) 50p. [84]

Tate and Lyle. Group Research and Development Annual Report 1975. Pp. 40. (Whiteknights, Reading: Tate and Lyle, Group Research and Development, 1976.) [94]